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PB86-117942

Drinking Water Criteria Document for Xylenes (Final Draft)

(U.S.) Environmental Protection Agency
Cincinnati, OH

Mar 85

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

2.

3. RECIPIENT'S ACCESSION NO. PB86 117942 /AS

SUBTITLE
Drinking Water Criteria Document for
Xylene

5. REPORT DATE 3/85

6. PERFORMING ORGANIZATION CODE

HOR(S)
Environmental Protection Agency

8. PERFORMING ORGANIZATION REPORT NO.

PERFORMING ORGANIZATION NAME AND ADDRESS

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Office of Drinking Water (WH-550)
401 M St., S.W., Washington, D.C. 20460

13. TYPE OF REPORT AND PERIOD COVERED Scientific review

14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on xylenes. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of xylenes
- Toxicokinetics and human exposure to xylenes
- Health Effects of xylenes in humans and animals
- Mechanisms of toxicity of xylenes
- Quantification of toxicological effects of xylenes

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Xylene properties Xylene exposure Xylene toxicity Drinking Water		

18. DISTRIBUTION STATEMENT OPEN	**19. SECURITY CLASS** *(This Report)* Non-sensitive	**21. NO. OF PAGES** 194
	20. SECURITY CLASS *(This page)* Non-sensitive	**22. PRICE**

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

United States
Environmental Protection
Agency

FINAL DRAFT
ECAO-CIN-416
March, 1985

EPA-600/X-84-185-1

PB86-117942

EPA

Research and Development

DRINKING WATER CRITERIA DOCUMENT FOR XYLENES

Prepared for

OFFICE OF DRINKING WATER

Prepared by

Environmental Criteria and
Assessment Office
Cincinnati OH 45268

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NOTICE

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LIST OF ABBREVIATIONS

AADI	Adjusted acceptable daily intake
ADI	Acceptable daily intake
BUN	Blood urea nitrogen
CNS	Central nervous system
EEG	Electroencephalogram
HA	Health advisory
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
ppm	Parts per million
SCE	Sister chromatid exchange
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
TLV	Threshold limit value
TWA	Time-weighted average
VER	Visual evoked response
W	Watts

I. SUMMARY

In surface waters, xylene volatilization appears to be the dominant removal process, as predicted by an EXAMS model. The volatilization half-lives calculated by the model range from 2.6-11.2 days. Oxidation does not appear to be significant, nor does photolysis, since the xylenes do not absorb light significantly >300 nm.

Sorption will occur, accounting for varying accumulations in the sediments (4.5-70% of the total xylene load from the EXAMS model).

Biodegradation may be significant, but appears to be highly variable. The variability may be due to the toxicity of the xylenes to microorganisms at high feed rates. Much monitoring data is available, however, that identifies the xylenes in drinking water, both from the tap and at the source. This indicates that the xylenes are degraded poorly to moderately in water.

Xylene is a dimethylbenzene and occurs in three position isomers: o-, m- and p-xylene. Mixed xylene is usually a mixture of the isomers and ethylbenzene. Xylene is a colorless, volatile liquid that is used as a lipid solvent. The half-life of xylene in water is ~6 hours, depending on depth, and loss is primarily by evaporation. Xylenes are soluble in water at ~175-200 mg/ℓ at environmental temperatures, and the log octanol/water partition coefficients for the isomers range from 2.77 (o-xylene) to 3.20 (m-xylene).

Absorption data on xylene are very limited but absorption can be inferred to be complete from the available metabolism and excretion studies. These studies show that ~85-95% of the orally administered dose is accounted for as urinary metabolites, while ~5% is expired through the lungs. Absorption following inhalation of xylene is comparable in animals and humans and is usually about 60% efficient. Dermal absorption is usually minor following exposure to xylene vapor, but may represent a significant route if liquid xylene contacts the skin. Distribution of xylene throughout the body following absorption is usually rapid and complete. Bioaccumulation of xylene seems likely in adipose tissue and possibly tissues of high lipid content such as myelin. Accumulation of xylene after extended periods of exposure may actually be lower than after short-term exposures because of more efficient hepatic metabolism of xylene. In animals, m- and p-xylene are predominantly metabolized to m- and p-methylhippuric acid, while o-xylene is metabolized to o-methylhippuric acid and o-toluyl glucuronide. In humans, all isomers seem to be metabolized to their respective methylhippuric acids. Aromatic hydroxylation represents only a minor metabolic route. Nearly 95% of absorbed xylene is excreted in the urine, while the remainder usually is exhaled from the lungs. Urinary excretion of metabolites is described as a three-phase process, with the slowest phase having a half-life of ~20-30 hours postexposure.

The different xylene isomers have slightly different toxicities and lethalities in laboratory animals, but none can be considered the most potent because their potencies vary from report to report. Generally, LD_{50} values for oral ingestion range from 3.6-6.75 g/kg for the isomers and slightly higher for mixed xylene. The LC_{50} values for xylene inhalation are ~6500 ppm for 4 hours. Based on acute oral toxicity data, a dose of ~1.0 g/kg/day is near the LOAEL in rats. A level of ~575 ppm (2500 mg/m³) was near the NOAEL for 4 hours of inhalation in rats. A higher level, however, near 3500 mg/m³ for 6 hours/day, 5 days/week could be tolerated with no apparent effects for up to 13 weeks. Subchronic ingestion of xylene in the diet at 200 mg/kg food for up to 6 months caused intracellular hepatocyte changes in rats, but no adverse toxic effects. Chronic toxicity testing has been conducted only for xylene inhalation. A level near 1000 ppm for 8 hours/day, 7 days/week for up to 1 year caused hepatotoxicity and lower body weight in exposed rats. This exposure level was considered a LOAEL.

The organs affected by xylene are the nervous system, lungs, liver and kidneys: the nervous system and lungs are usually affected after acute exposure; and the liver and kidneys are affected by the longer-term, low-level xylene exposure. Nervous system effects include incoordination, decreased balance control, prostration and narcosis. Pulmonary effects include irritation, congestion, edema and hemorrhage. Hepatic enzymes are induced and hepatomegaly is noted after xylene exposure, but severe hepatic necrosis or toxic effects have not been shown. Renal effects have not been microscopically verified.

The single carcinogenicity test on xylene applied the compound to the skin of mice and reported xylene as a noncarcinogen. A 2-year bioassay on xylene, administered by gavage, is in progress but preliminary or final data are not available. Xylene has not been shown to be a mutagen in Ames Salmonella assays or in E. coli mutant reversion assays. Xylene does appear to be embryotoxic and maternotoxic, and usually causes delayed fetal development and skeletal maturation. Increased incidences of cleft palates have been reported in fetuses exposed intraplacentally at oral doses of 20 g/kg/day.

A number of experimental studies have been conducted on the toxicity of xylene in humans. A level of near 100 ppm (434 mg/m³) for ~8 hours/day for up to 5 days appears to be near a no-effect level for eye and throat irritation. More sensitive individuals and females may report slight effects at this level. At levels near 300-400 ppm (1300-1730 mg/m³), reaction time, body balance and dexterity become affected. Case reports on painters, hospital workers and histology laboratory workers have been reviewed. One study suggested illness in hospital workers within 0.25-1.25 hours after exposure to xylene at levels estimated to be 700 ppm (3040 mg/m³). Occupational exposure to industrial solvents including xylene has been suggestively associated with renal tubular and glomerular damage and decrement of neuromuscular function. These studies can only suggest xylene as a possible etiologic agent since exposure levels are rarely available and exposures are usually to several chemicals simultaneously. The number of workers exposed to xylene is great and all are at higher risk of intoxication. Pregnant women and their fetuses should be regarded as a group highly susceptible to xylene toxicity.

Most of the toxic effects of xylene are attributable to the parent compound, but minor metabolites, methylbenzaldehyde and arene oxides have been suggested as toxic intermediates. Effects on the CNS may stem from physical toxicity, which is due to this system's sensitive physiology, its high lipid content and xylene's lipophilic nature. Solvation or fluidization of the axonal membrane may also cause impaired nervous function. Certain central nervous pathways, such as those controlling body balance in the central vestibular region, may be more sensitive and affected at lower xylene levels than other nervous pathways. Pulmonary and renal effects appear to be attributable to endothelial and epithelial irritation or altered permeability. These changes could lead to hemorrhage in the lungs and altered renal filtration. Effects in the liver have been described as functional adaptation to increased xylene metabolism. Severe hepatic effects have not been shown. A large number of studies have shown xylene's inductive effect on hepatic enzymes and a few studies have shown ultrastructural evidence of increased hepatic metabolic potential. Synergistic effects of xylene concomitant with other chemicals stem from metabolic interactions. Ethanol potentiates xylene toxicity by delaying its metabolism and elevating blood-xylene levels. The hepatotoxicity of carbon tetrachloride is potentiated by xylene through enzyme induction, causing an increase in the active metabolite of carbon tetrachloride. Toluene and xylene appear to interact equally, causing metabolism of each to be slightly delayed during simultaneous exposure. Benzene and ethylbenzene metabolism is delayed by simultaneous xylene treatment.

The appropriate toxicity data in laboratory animals and humans pertinent to the quantification of a drinking water HA level have been reviewed. The 1-day HAs for an adult and a child were determined based on an acute human inhalation NOAEL. These values are 42 and 12 mg xylene/ℓ, respectively.

The 10-day HA values were determined from a NOEL in rats exposed by inhalation. The values for an adult and a child are 27 and 7.8 mg/ℓ, respectively. The lifetime HA or AADI was based on a NOEL in rats, monkeys, dogs and guinea pigs inhaling xylene continuously for 90 days. The calculated AADI was 2.2 mg xylene/ℓ.

An estimate of carcinogenic risk could not be made because of lack of carcinogenicity data for xylene.

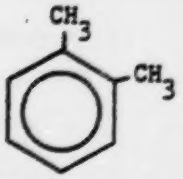
Special groups at elevated risk of xylene intoxication include workers exposed to xylene and pregnant women and their fetuses. Possibly at greatest risk are those workers exposed to xylene and other compounds that act synergistically. The synergistic effect of ethanol, in the form of alcoholic beverages, with xylene may be of primary concern since simultaneous exposure to these two compounds may be common.

II. GENERAL INFORMATION AND PROPERTIES

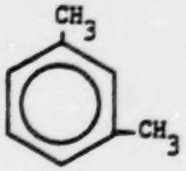
Structure and Identification

Commercial xylene is a mixture of the three isomers, o-, m- and p-xylene, the m-isomer predominating (Windholz, 1983). Synonyms for xylenes are xylols and dimethylbenzenes.

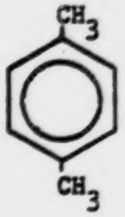
o-Xylene is 1,2-dimethylbenzene. Its structure follows:



m-Xylene is 1,3-dimethylbenzene. Its structure follows:



p-Xylene is 1,4-dimethylbenzene. Its structure follows:



The molecular formula for the three xylenes is C_8H_{10}, and the molecular mass for each is 106.16. The Chemical Abstracts Service (CAS) registry numbers and the Registry of Toxic Effects of Chemical Substances (RTECS) numbers for the xylenes are shown in Table II-1.

TABLE II-1

CAS and RTECS Numbers for the Xylenes

Chemical	CAS Number	RTECS Number*
Xylenes	1330-20-7	ZE 2100000
o-xylene	95-47-6	ZE 2450000
m-xylene	108-38-3	ZE 2275000
p-xylene	106-42-3	ZE 2625000

*Registry of Toxic Effects of Chemical Substances, 1980

Physical and Chemical Properties

The physical properties of the xylenes are summarized in Tables II-2, II-3, II-4 and II-5.

All the xylenes are colorless liquids with a mild characteristic sweet odor.

Stability in Water

Based on an EXAMS model (Burns et al., 1981), the dominant process for removal of xylenes in water will be volatilization. Input parameters for the model were taken from Tables II-1 through II-5 and Hendry et al. (1974). The predicted volatilization rates are as follows:

	Volatilization half-life (days)
o-xylene	2.6-11
m-xylene	2.8-11
p-xylene	2.7-11

Rates will vary depending on the type of environment, temperature, oxygen exchange rate, and amount of organic matter in the sediments.

Oxidation by peroxy radical accounted for <1% of the total load in the EXAMS model (Burns et al., 1981) when the rate constants reported by Hendry et al. (1974) were used. The model uses a peroxy radical concentration of 0.5 to 1×10^{-9} M, depending on the compartment. Since the xylenes do not absorb light significantly >300 nm, photolysis will not occur (Weast, 1972).

TABLE II-2

Xylenes*

Property	Description
Boiling point	137-140°C
Density	0.86
Flash point	29°C
H_2O solubility	practically insoluble in H_2O
Solubility in organic solvents	miscible with absolute alcohol, ether and many other organic liquids

*Source: Windholz, 1983

TABLE II-3

o-Xylene

Property	Description	Reference
Boiling point	144.4°C 139.3°C	Verschueren, 1983 Windholz, 1983
Conversion factor	1 mg/m³ = 0.23 ppm 1 ppm = 4.41 mg/m³	Verschueren, 1983
Flash point	17°C; closed cup	Windholz, 1983
H_2O solubility	175 mg/ℓ at 20°C insoluble in water	Verschueren, 1983 Windholz, 1983
Log octanol/water partition coefficient	3.12	Hansch and Leo, 1979
Melting point	-25°C	Verschueren, 1983
Refraction index	1.4973 at 20°C; sodium light	Windholz, 1983
Solubility in organic solvents	soluble in alcohol, ether	Windholz, 1983
Specific gravity	0.88, at 20°C, referred to water at 4°C	Verschueren, 1983
Vapor pressure	5 mm Hg at 20°C	Verschueren, 1983

TABLE II-4

m-Xylene

Property	Description	Reference
Boiling point	139°C	Verschueren, 1983
Conversion factor	1 mg/m³ = 0.23 ppm 1 ppm = 4.41 mg/m³	Verschueren, 1983
Flash point	25°C, closed cup	Windholz, 1983
H_2O solubility	160 mg/ℓ at 20°C	Chernoglazova and Simulin, 1976
	insoluble	Windholz, 1983
Log octanol/water partition coefficient	3.20	Verschueren, 1983
Melting point	-48/-53°C -47.4°C	Verschueren, 1983 Windholz, 1983
Refraction index	1.4973 at 20°C; sodium light	Windholz, 1983
Solubility in organic solvents	soluble in alcohol, ether and many organic solvents	Windholz, 1983
Specific gravity	0.864 at 20°C	Verschueren, 1983
Vapor pressure	6 mm at 20°C	Verschueren, 1983

TABLE II-5

p-Xylene

Property	Description	Reference
Boiling point	138.4°C 137-138°C	Verschueren, 1983 Windholz, 1983
Conversion factor	1 mg/m³ = 0.23 ppm 1 ppm = 4.41 mg/m³	Verschueren, 1983
Flash point	25°C, closed cup	Windholz, 1983
H_2O solubility	198 mg/ℓ at 25°C	Verschueren, 1983
Log octanol/water partition coefficient	3.15	Verschueren, 1983
Melting point	13°C 13-14°C	Verschueren, 1983 Windholz, 1983
Refraction index	1.49575 at 20°C, sodium light	Windholz, 1983
Solubility in organic solvents	soluble in alcohol, ether and many other organic solvents	Windholz, 1983
Specific gravity	0.86 at 20°C	Verschueren, 1983
Vapor pressure	6.5 mm Hg at 20°C	Verschueren, 1983

Sorption to sediments had a varying effect, yielding a total steady-state accumulation of 4.5-70% in bottom sediments, depending on the percent organic matter. Green et al. (1981) found that the movement of o- and p-xylene through two clays and Ranger shale was inversely related to the octanol/water partition coefficient. Movement increased as the bulk density of the soil decreased. Nathwani and Phillips (1977) found similar results for o-xylene, with the Freundlich K varying between 11.03 for a silty clay with 16.2% organic content and 0.617 for a silty loam with 1% organic content.

Biodegradation varied considerably for the three xylenes, depending on the source of the seed and whether or not it was acclimated. Bridie et al. (1979) found 52, 80 and 44% of theoretical biochemical oxygen demand (BODT) in 5 days for o-, m- and p-xylenes, respectively, using a filtered sewage seed. Acclimation increased degradation to 51% for o-xylene and 74% for p-xylene. Using very high feed rates of 500 mg/ℓ, Malaney (1960), Marion and Malaney (1964) and Malaney and McKinney (1966) found m-xylene resistant to degradation (toxic to microorganisms), yielding >10% of the BODT after 8 days. o-Xylene and p-xylene were more degradable, varying between 63 and 26% of the BODT. Surprisingly, Kappeler and Wuhrmann (1978) found complete degradation of all three xylenes in 8 days in groundwater containing 300-400 cells/mℓ. The xylenes were contained in gas oil percolated through a column packed with quartz sand at 10°C. The system had a 2-hour detention time. Prior to the experiment, the system was flushed for 4 days with groundwater to remove any microorganisms that would yield spurious results. Dore et al. (1975) found only 1% BODT for p-xylene in 5 days using three polluted surface waters as the inoculum. Based on these results, with the exception of the report by Kappeler and Wuhrmann (1978), it can be concluded that the xylenes are only poorly to moderately degraded in most systems. Relatively high feed rates were used by some of the studies reported, however, which may account for the low degradation rate from toxicity.

The poor to moderate degradation rate is supported by many reports of xylenes in drinking water. Several of these reports are summarized in Table II-6. Since these show xylene concentrations in both groundwater and surface water, degradation in both media appears to be similar.

Summary

In surface waters, volatilization appears to be the dominant removal process as predicted by an EXAMS model (Burns et al., 1981). The volatilization half-lives calculated by the model range from 2.6-11.2 days. Oxidation does not appear to be significant (Hendry et al., 1974), nor does photolysis, since the xylenes do not absorb light significantly >300 nm (Weast, 1972).

Sorption will occur, accounting for varying accumulations in the sediments (4.5-70% of the total xylene load from the EXAMS model of Burns et al., 1981) (Green et al., 1981; Nathwani and Phillips, 1977).

Biodegradation may be significant, but appears to be highly variable (Bridie et al., 1979; Malaney, 1960; Malaney and McKinney, 1966; Marion and Malaney, 1964; Kappelar and Wuhrmann, 1978; Dore et al., 1975). The variability may be due to the toxicity of the xylenes to microorganisms at high feed rates. Much monitoring data are available, however, which identify the

TABLE II-6

Xylene Concentrations in Drinking Water

Location	Water Type	Concentration ($\mu g/\ell$)	Xylene Isomers	Reference
Washington, DC	tap	NQ	o-, m-, p-	Saunders et al., 1975
New Orleans	drinking	0.33-3.4	o-	Keith et al., 1976
Mississippi	river and Artesian well	NQ	o-	Dowty et al., 1975
Philadelphia	drinking	NQ	o-, m-, p-	Suffet et al., 1976
Great Lake Municipalities	drinking supplies	0-9.9	o-, m-	Williams et al., 1982
Canada	drinking	0-2	o-, m-	Otson et al., 1982
Tuscaloosa	drinking	NQ	o-, p-	Bertsch et al., 1975
Houston	drinking	NQ	o-, p-	Bertsch et al., 1975

NQ = Not quantified

xylenes in drinking water, both from the tap and at the source (Saunders et al., 1975; Keith et al., 1976; Suffet et al., 1976; Otson et al., 1982). This indicates that the xylenes are degraded poorly to moderately in water.

III. TOXICOKINETICS

Aspects of the absorption, distribution, bioaccumulation and retention, metabolism, and excretion of xylene in experimental animals and humans will be reviewed in this section. Where appropriate and when data are available, the specific isomers of xylene will be noted; otherwise "xylene" may refer to a mixture of the isomers or an unspecified isomer.

Absorption

Data on the absorption efficiency of xylenes from the gastrointestinal tract of animals or humans were not located. It can be inferred, however, that absorption by this route is nearly complete, since Bray et al. (1949) noted that 85-90% of the administered dose of isomers was accounted for in the urine while pulmonary excretion may have accounted for the remaining xylene (see Metabolism and Excretion Sections).

The available studies on xylene absorption in man are concerned primarily with inhalation and dermal exposure since these routes are occupationally important. Astrand et al. (1978) reported a study on 12 male volunteers who were exposed by inhalation to mixed xylene (49.4% m-xylene, 8.8% o-xylene, 1.4% p-xylene and 40.4% ethylbenzene) through a breathing valve. Each subject was exposed for four 30-minute periods at rest or on a bicycle ergometer. Six men were exposed at 870 mg/m³ (200 ppm) for 30 minutes of rest followed by 90 minutes (3x30 minutes) of exercise at 50 W. The remaining six men were exposed at 435 mg/m³ (100 ppm) mixed xylene for 30 minutes each at rest, 50 W, 100 W and 150 W exercise. Heartbeat and ventilatory rate and volume were monitored. At rest and during light work

(50 W), pulmonary uptake of xylene was about 63% over the 2-hour exposure period. More strenuous exercise caused an increase in the relative amount of xylene taken up, but correction for increased pulmonary exchange showed that uptake efficiency gradually decreased from 63-64% at rest and 50 W exercise to 51% at 150 W exercise (Astrand et al., 1978).

Six male volunteers were exposed to m-xylene (laboratory grade) at 3.9 mmole/m³ (414 mg/m³) for 6 hours/day on 5 consecutive days and tested for xylene uptake (Riihimaki et al., 1979a). The final 3 hours of exposure on the fifth day was at 8.2 mmole/m³ (870 mg/m³). These subjects were exposed at rest in a chamber so that uptake was actually by inhalation and percutaneous absorption. Absorption of xylene ranged from 54-68%, averaging ~60%, and remained relatively constant throughout the exposure period. In a similar study, Riihimaki et al. (1979b) exposed 12 male volunteers to various levels of m-xylene (averaging 100-400 ppm) in combination with exercise. Exposure was 6 hours daily (3 hours in morning, 1 hour lunch break with no exposure, 3 hours afternoon exposure) on 5 successive days. One group (n=4) was exposed to ~90 ppm m-xylene (390 mg/m³) on the first 4 days and 3 hours of the fifth day with m-xylene at 191 ppm (830 mg/m³) for the final 3 hours of each week, and was exercised on a bicycle ergometer (100 W) for four 10-minute periods each day. A second group of volunteers (n=8) was exposed to 70 ppm (304 mg/m³) with peak levels of 200 ppm every hour during the morning and afternoon exposures. The peak concentrations were 10 minutes in duration and 5 minutes was allowed for the rise and fall of m-xylene concentrations in the chamber. A 10-minute work period (100 W) coincided with each of the peak exposure periods (4 per day). On the afternoon (3 hours) of the fifth day, exposure was at 130 ppm (565 mg/m³) with two peak m-xylene concentrations at 400 ppm (1736 mg/m³). Absorption of m-xylene, measured as the difference between the chamber air concentration and the exhaled air concentration, was near 60% under all test conditions. At rest absorption ranged from 53-68%, ande during exercise absorption was consistently similar (55-71%).

Consistent with the absorption factors reported by Riihimaki et al. (1979a,b), Sedivec and Flek (1974, 1976) reported that humans exposed to o-, m-, p-xylene or a mixture (1:1:1) of the three by inhalation absorbed ~64%. Fifteen experiments, using four persons each, were conducted for 8 hours of exposure at 200 or 400 mg/m³. All isomers or the mixture were absorbed almost identically and apparently did not change at the different exposure levels or at different periods during the 8 hours of exposure. Individual differences did occur between subjects and appeared to be indirectly related to ventilation rate (decreased retention with increased ventilation).

In a study in which 15 male volunteers were exposed to mixed xylene at 435 and 1300 mg/m³ during rest and 1300 mg/m³ while exercising (100 W), Gamberale et al. (1978) reported mean absorption levels of 180, 541 and 1210 mg/70 minutes of exposure, respectively. Assuming a daily ventilatory volume of 20 m³ while at rest and 10 m³/8 hours while performing work, absorption efficiency was about 43, 43 and 64%, respectively. These values are corroborative with those reported by Riihimaki et al. (1979a,b) and Sedivec and Flek (1974, 1976).

Se'nczuk and Orlowski (1978) conducted laboratory tests with 5 men and 5 women exposed to 100, 300 and 600 mg/m³ m-xylene for 8 hours at rest. The average ventilation rates were 0.458 m³/hour for women and 0.575 m³/hour for men. The average amounts of m-xylene absorbed by women during 8 hours of exposure at 100, 300 and 600 mg/m³ were 272, 724 and 1359 mg, so that pulmonary retentions were 74, 66 and 62%, respectively. In men, the absorbed doses were 342, 909 and 1711 mg and the respective absorption coefficients were 74, 66 and 62%. Se'nczuk and Orlowski (1978) noted that retention was quite high initially but decreased throughout the exposure period.

Percutaneous absorption of xylenes in man, as evident from the following four studies (Riihimaki and Pfaffli, 1978; Engstrom et al., 1977; Riihimaki, 1979a; Dutkiewicz and Tyras, 1968), is quite low in comparison with pulmonary absorption. Riihimaki and Pfaffli (1978) exposed five male volunteers to 300 or 600 ppm (1300 or 2600 mg/m³) for 3.5 hours with respiratory protection (fullface respirators). For comparative purposes the subjects were also exposed at another time to xylene at 20 ppm for 3.5 hours. The volunteers were dressed in thin-clothed pajamas and socks. Amounts of xylene absorbed (determined by the amount excreted in urine and expired air) after percutaneous exposure at 300 and 600 ppm and inhalation exposure at 20 ppm were 21, 45 and 38 mg, respectively. Based on an assumption of 60% pulmonary absorption, Riihimaki and Pfaffli (1978) concluded that percutaneous absorption represented 1.3-1.4% of the xylene that would have been absorbed by the lungs at the same exposure levels. Based on the average skin surface area of subjects (1.88 m²), percutaneous absorption was 0.32 μg/cm²/hour for 300 ppm exposure and 0.68 μg/cm²/hour for exposure to 600 ppm m-xylene vapor.

Absorption of pure liquid m-xylene through the hands was studied by Engstrom et al. (1977) and Riihimaki (1979a). Subjects immersed both hands in pure (laboratory grade) m-xylene for 15 minutes and precautions were taken to minimize inhalation exposure. Absorbed xylene was determined to be that quantity excreted in the urine as methyl-hippuric acid within 22 hours after exposure. Riihimaki (1979a) reported that ~22.5 mg of m-xylene was absorbed within 15 minutes and the average area of the hands was near 900 cm^2 for both hands. The absorption rate was, therefore, near 100 μg/cm^2/hour. Engstrom et al. (1977) had estimated previously that ~35 mg of m-xylene was absorbed during a 15-minute exposure and the absorption rate was ~120 μg/cm^2/hour. This extreme dermal exposure was equated to an inhalation exposure at 100 ppm of equal duration.

Dutkiewicz and Tyras (1968) reported that dermal absorption of pure xylenes through the skin of the forearm was 4.5-9.6 mg/cm^2/hour. Ten volunteers had liquid xylene applied to the forearm under a sealed watch-glass for 1 hour. The xylene absorbed through the skin was that amount not recovered after several ethanol washings of the skin. The higher rate of absorption reported here compared with levels summarized previously (Riihimaki and Pfaffli, 1978; Riihimaki, 1979a) may be due to evaporative loss, loss during washing or the absorptive difference between the hands (thick skin) and the forearm (thin skin).

The rate of o-xylene penetration through excised rat skin was determined _in vitro_ by Tsuruta (1982). Abdominal skin of rats was depilated and mounted in a diffusion cell with o-xylene placed on the exterior and 0.9% NaCl solution as collection fluid on the interior. The area of the skin was

2.55 cm². Exposure to o-xylene was for 3-6 hours at 25°C and the amount of compound collected in the saline solution was determined by gas chromatography. Based on 6 to 9 experiments at each exposure duration, the amount of o-xylene penetrating the skin was 10.4, 18.8, 34.3, 41.1 and 59.4 μg, respectively, after 3, 3.5, 4, 5 and 6 hours. Based on these data, Tsuruta (1982) reported a penetration rate of about 6.2 μg/cm²/hour and a lag time of 2.2 hours for o-xylene penetration.

Distribution

Using whole-body autoradiography to detect radiolabeled xylene and metabolites, Bergman (1978) reported distribution of the compound in many tissues and organs of exposed mice. ^{14}C-m-Xylene was administered to mice by inhalation for 10 minutes and whole mice were embedded, frozen and sectioned before exposure to X-ray film. In mice sacrificed immediately after xylene exposure, radiolabel was detected in the lungs, liver and kidneys. Rapid distribution to the brain and adipose tissue was also evident. Two hours after exposure, radiolabel in the lungs was restricted to the bronchi. In addition to the previously noted organs, radiolabel was detected in the intestine after 2 hours. The last traces of xylene were detected in the adipose tissue of mice sacrificed 4 hours after exposure (Bergman, 1978).

Nawrot et al. (1980) reported in an abstract that maximal distribution of ^{14}C-m-xylene to the liver of pregnant CD-1 mice occurred at 2 hours after exposure. Mice were treated by gavage (dose not specified) on day 12 or 15 of gestation. Radioactivity, reportedly from metabolites of m-xylene, was detected in fetal tissues, maximally 2 hours after dosing.

In rats exposed to ^{14}C-p-xylene for 1-8 hours by inhalation at 208 mg/m^3 (48 ppm), radiolabel was detected (in decreasing concentration) in the kidneys, subcutaneous fat, ischiadic nerve, blood, liver, lungs, spleen, muscles, cerebrum and cerebellum (Carlsson, 1981). Distribution to all tissues was rapid, allowing near maximal levels in tissues (except in kidneys and subcutaneous fat) within 1 hour.

Few data on the distribution of xylene in humans are available. Riihimaki et al. (1979a) noted that xylene, distributed by the blood, is predominantly associated with serum proteins (90%) and only a small fraction (10-15%) exists in the serum. Engstrom and Riihimaki (1979) studied the distribution of m-xylene to the subcutaneous fat in 18 male volunteers exposed to xylene at 64-400 ppm (278-1740 mg/m^3) for 6 hours/day for up to 6 days. Subcutaneous biopsies of gluteal adipose tissue were taken for xylene determination. This study showed that distribution and accumulation of xylene (see Bioaccumulation and Retention Section) did occur in the adipose tissue.

Bioaccumulation and Retention

Data available on the accumulation of xylene indicate that the adipose tissue is the predominant site of retention in laboratory animals and humans. The xylene accumulated in the adipose tissue, however, may represent only about 5-10% of the total absorbed dose (Engstrom and Riihimaki, 1979).

In rats exposed to ~48 ppm (208 mg/m^3) ^{14}C-p-xylene by inhalation for 1, 2, 4 or 8-hours, concentrations in subcutaneous fat were 8.1, 13.2, 19.3 and 28.7 µg/g tissue (Carlsson, 1981). At 1 and 3 hours after 8 hours of exposure, xylene levels in the subcutaneous fat were 27.6 and 21.4 µg/g tissue, respectively. Levels of xylene in the kidney (114.7 µg/g tissue) were 4-5 times greater than that in the subcutaneous fat during exposure but dropped more rapidly after exposure was terminated. Much of the radiolabel detected in the kidney was probably due to xylene metabolites in the renal filtrate and not cellular accumulation. In the subcutaneous fat, however, unreacted xylene was the major radioactive component detected (Carlsson, 1981).

In male Wistar rats exposed to 300 ppm (1300 mg/m³) xylene (80% m-xylene, 12% p-xylene) for 6 hours/day on 5 days/week for 2 weeks, accumulation of the solvent in the perirenal fat was evident (Savolainen et al., 1978). Perirenal fat samples from rats sacrificed after the fifth exposure contained a mean of 64.8 µg xylene/g tissue. Samples taken after 10 exposures contained 127.0 µg xylene/g perirenal adipose tissue. In a similar study using male Wistar rats exposed by inhalation to 50, 400 or 750 ppm in xylene (215, 1735 or 3250 mg/m³), Savolainen and Pfaffli (1980) reported accumulation of the solvent in the perirenal fat and brain tissue. Accumulation was most notable when comparing xylene-tissue levels from rats treated at the same exposure level for 1 week versus 2 weeks (Table III-1). A linear relationship between exposure level and tissue concentration was shown and this relationship also indicated accumulation of xylene in both tissues from week 1 to 2 (Savolainen and Pfaffli, 1980). Based on data of the sequestration of m-xylene in the fat, Elovaara (1982) suggested that exposure at 750 ppm (6 hours/day, 5 days/ week) represented the level of metabolic saturation in rats exposed for 2 weeks. The accumulation of

TABLE III-1

Levels of m-Xylene in Brain and Perirenal Fat of Exposed Rats[a]

Exposure Level ppm (mg/m^3)[b]	Brain Levels (μg/g)[c]		Perirenal Fat Levels (μg/g)[c]	
	Week 1	Week 2	Week 1	Week 2
50 (215)	0.66	1.0	15.1	27.8
400 (1735)	5.7	8.8	150.1	200.9
750 (3250)	10	13.2	311.2	474.6
Linear relationship between tissue level and exposure level[d]	$y = 3.17x + 0.88$	$y = 4.04x + 7.00$	$y = 98.56x - 94.45$	$y = 146.77x - 191.53$

[a] Source: Savolainen and Pfaffli, 1980

[b] Exposure was for 6 hours/day, 5 days/week; originally μmol/ℓ.

[c] Tissue levels determined by gas chromatography of samples from 5 animals at each exposure/duration level. Originally nmol/g.

[d] Relationship where x = exposure level (μmol/ℓ) and y = tissue m-xylene concentration (nmol/g).

xylene in the fat with respect to dose and duration (tissue levels after 1 and 2 weeks of exposure) followed a nonlinear trend toward increased accumulation following exposure at 750 ppm.

The accumulation and levels of xylene in the perirenal fat of rats exposed for a subchronic period was studied by Savolainen et al. (1979). Twenty male Wistar rats were exposed to 300 ppm technical xylene (85% m-xylene, 15% other isomers) for 6 hours/day, 5 days/week for 5-18 weeks. Five animals each were sacrificed after 5, 9, 14 and 18 weeks of exposure and perirenal fat was removed and analyzed for xylene content. Mean xylene levels (measured as m-xylene by gas chromatography) were 67.6, 57.4, 40.7 and 36.6 μg/g tissue, respectively. Xylene apparently accumulated in the fat before the 5-week study and decreased gradually as exposure was continued for longer periods. The decrease in solvent load, even though xylene exposure continued, may have been caused by increased metabolic rate (Savolainen et al., 1979).

The accumulation of mixed xylene in humans was studied in 12 male volunteers exposed at 100 or 200 ppm (435 or 870 mg/m^3) by inhalation for 2 hours (Engstrom and Bjurstrom, 1978). Six subjects were exposed to 200 ppm mixed xylene (49.4, 8.8 and 1.4%, m-, o- and p-xylene and 40.4% ethylbenzene) for 30 minutes at rest and for 90 minutes exercising at 50 W of work. A second group of 6 subjects was exposed at 100 ppm for four 30-minute intervals of rest and work at 50, 100 and 150 W, respectively. In both groups, exposure was interrupted for 20 minutes after the initial rest period before exercise. Adipose tissue biopsies from the subcutaneous layer in the gluteal area were taken at 0.5, 2, 4 and 22 hours after the termination of exposure. The uptake and accumulation of xylene was proportionately related to the amount of body fat of the subjects. The concentration of m-xylene, p-xylene and ethylbenzene (o-xylene could not be separated in the chromatographic column used) in adipose tissue was dose-dependent. In subjects exposed at 200 ppm, mean tissue levels were 8, 9, 6 and 6 mg/kg after 0.5, 2, 4 and 22 hours, while levels in subjects exposed at 100 ppm were 4, 6, 4 and 8 mg/kg, respectively. Engstrom and Bjurstrom (1978) noted that the 4-hour values were based on only 4 subjects and should be viewed with caution. Since there was no significant change in the concentration of xylenes in adipose tissue from 2-22 hours postexposure, the elimination of the solvent from this tissue was regarded as slow and accumulation after repeated exposure is possible.

In a subsequent study, Engstrom and Riihimaki (1979) exposed 18 male volunteers to m-xylene by inhalation at 64-400 ppm (278-1740 mg/m^3) on various daily exposure schedules for 6 days. Three different exposure patterns were used. Exercise (bicycle ergometer) coincided with peak levels of exposure (up to 400 ppm) in one. The second included constant xylene concentrations with exercise, while the third pattern used varied exposure levels without exercise. The uptake of m-xylene to adipose tissue was greatest in subjects who were exercising during high levels of exposure (8.0% of total absorbed dose; 20.6 mg/kg fat), while exercise at constant exposure levels caused moderate uptake (7.5%; 17.7 mg/kg fat). Subjects exposed while at rest showed the least uptake of xylene in the adipose tissue (3.7%; 9.5 mg/kg fat). The half-life time of m-xylene in the adipose tissue was 77.5, 47.5 and 49.5 hours, respectively, for the three dose scheddules. It is important to emphasize that exposure to m-xylene at ~100 ppm (TLV level) for 6 hours/day, simulating occupational exposure, results in the retention of xylene in adipose tissue and the half-life was 47.5 hours, so that daily and week-to-week accumulation is possible. Corroboratively, Riihimaki et al. (1979a) cited a distribution coefficient for xylene between adipose tissue and blood of 97:1 and suggested a persistence time of ~67 hours for this tissue.

Metabolism

As will be evident from data reviewed in this section, the three isomers of xylene (o-, m- and p-xylene) are metabolized by slightly different pathways, which results in different proportions of metabolites. Generally 85-95% of the absorbed xylene is metabolized and excreted in the urine. Only a small amount (5-10%) is unmetabolized and exhaled through the lungs. It is also evident after comparison of the available data that metabolism of the xylenes in laboratory animals is somewhat different from that in humans.

As reviewed by Browning (1965), the oxidation of one of the methyl groups of xylene, producing toluic acid and subsequent glycine conjugation, has been considered the major metabolic pathway for over a century. More recent research has quantitatively studied the metabolism of the different xylene isomers and has identified minor metabolites.

The urinary metabolites of o-, m- and p-xylene following oral administration (in olive oil) were determined in rats, guinea pigs and rabbits (Fabre et al., 1960). Animals were treated by gavage at 40 mg/kg/day for 1 week. Toluic acid was a urinary metabolite following treatment with all isomers. Additionally, animals ingesting o-xylene excreted 3,4-dimethylphenol and those receiving p-xylene excreted 2,5-dimethylphenol and unchanged p-xylene. Quantitative data on the urinary metabolites in each of the species tested were not available, but the metabolites identified from all three species were comparable.

In a report based on original research in rats, guinea pigs and rabbits, and a review of previous metabolism studies on xylenes, Laham (1970) reported that all xylene isomers are oxidized to toluic acid, which is conjugated with glycine to form methylhippuric acid. The respective isomers of toluylglucuronic acid were reported after exposure to all three isomers, but the percentages of these toluic acid conjugates in contrast to methylhippuric acid were not reported. Aromatic hydroxylation does occur to a lesser extent to produce isomeric xylenols. The xylenols identified following o- and p-xylene exposure were 3,4- and 2,5-xylenol, respectively. m-Xylene could theoretically be metabolized to three isomeric xylenols, but none were detected. The xylenols are conjugated and excreted as glucuronides or sulfate esters.

Bakke and Scheline (1970) also reported phenolic metabolites (aromatic hydroxylation) of o-, m- and p-xylene following oral administration in rats. Male albino rats were maintained on a neomycin sulfate-containing diet for 7 days, while "control" urine samples were taken to determine background urinary phenols. On the seventh day, three rats each were treated by gavage with o-, m- or p-xylene at 100 mg/kg in propylene glycol. Urine samples collected over the subsequent 48 hours were analyzed by gas chromatography and compared with standards. Phenolic metabolites of o-xylene were 3,4-dimethylphenol (0.1% of administered dose) and 2,3-dimethylphenol (0.03%), while 2,4-dimethylphenol (0.9%) and 2,5-dimethylphenol (1.0%), respectively, were identified following m- and p-xylene treatment.

The metabolism of m- and o-xylene to phenolic metabolites was studied in male Wistar rats treated orally with 0.9 g/kg (Mikulski et al., 1979a). Blood and urinary phenols were determined at intervals from 0.5-36 hours after treatment. Maximal blood phenol levels were reached within the first half-hour of the study, reaching ~8 and 13 μg/mℓ, respectively, for m- and o-xylene. Urinary phenol excretion was greatest at 9 hours after treatment and reached rates <0.1 mg/hour. For m- and o-xylene, urinary excretion of phenol represented 0.8 and 0.7% of the administered dose, respectively.

The metabolic pathway of xylene to mercapturic acid in the rat was studied by Van Doorn et al. (1981). These thioester conjugates represent 2.4 and 1.3% of the administered dose (106 mg/kg intraperitoneally) of m- and p-xylene, respectively, but accounted for 16% of the o-xylene administered to rats. The metabolism of o-xylene to o-methylbenzylmercapturic acid entails initial oxidation to o-methylbenzyl alcohol, sulfation and finally conjugation of o-methylbenzyl sulfate with glutathione. Van Doorn et al. (1980) reported that liver glutathione levels were decreased to 26% of pretreatment levels after o-xylene treatment. Van Doorn et al. (1981) noted that o-methylbenzyl sulfate *in vitro* was ~20 times more reactive in combining with glutathione than were m- or p-methylbenzyl sulfate. This apparently would lead to higher levels of o-methylbenzylmercapturic acid compared with that for the m- and p-isomers.

The profile of metabolites of m-xylene has been found to be dose-dependent. Ogata and Fujii (1979) reported that urinary excretion of m-methylhippuric acid (glycine conjugate of m-toluic acid) accounted for 74.8, 65.4 and 53.5% of the m-xylene administered subcutaneously at 87, 174 and 348 mg/kg, respectively, in male Wistar rats.

Consistent with data reported by Van Doorn et al. (1981) on the metabolism of the xylene isomers in the rat, Bray et al. (1949, 1950) noted that o-xylene was metabolized in a manner different from m- or p-xylene in rabbits. Rabbits were treated orally with o-xylene at 0.9, 1.35 or 1.8 g/animal, m-xylene at 1.74 g/animal, or p-xylene at 1.7 g/animal. Urine was collected for 3 days postexposure. The urinary metabolites expressed as percent of administered dose for the three xylene isomers are summarized in Table III-2. It is evident from these data that o-xylene is readily oxidized and conjugated to the glucuronide of toluic acid. About 30% of the administered o-xylene is metabolized to this product, while 2 or 1% of the m- and p-xylene is excreted in this form. The m- and p-isomers are predominantly excreted as their respective methylhippuric acid metabolites. Aromatic hydroxylation to dimethylphenol occurs to a small degree for all isomers administered to rabbits (Bray et al., 1950).

The metabolism of p-xylene by rabbit liver and lung *in vitro*, has been studied by two research groups (Carlone and Fouts, 1974; Smith et al., 1982). Carlone and Fouts (1974) used liver and lung microsomal enzyme preparations to elucidate the metabolism of p-xylene. The major metabolite produced by the liver and lung microsomal preparations was p-toluic acid.

TABLE III-2

Urinary Metabolites of o-, m- and p-Xylene in Rabbits[a]

	Percent of Administered Dose[b]				
Compound	Oral Dose (g/animal)	Methyl-hippuric Acid[c]	Toluyl Glucuronide[d]	Mercapturic Acid[e]	Dimethyl-phenol
o-xylene	0.90	30	22	NR	NR
	1.35	36	29	8	2
	1.80	22	32	6	NR
m-xylene	1.74	79	2	4	4
p-xylene	1.70	87	1	0	2

[a] Source: Bray et al., 1949, 1950

[b] Collected over 3 days postexposure

[c] Isolated as "Ether-soluble acid"

[d] Isolated as "Ester glucuronide"

[e] Isolated as "Ethereal glucuronide sulfate"

NR = Not reported

The rate of toluic acid formation by lung microsomes plus lung soluble fraction was about half the rate for liver microsomes plus liver soluble fraction. The rate of toluic acid formation by lung microsomes plus liver soluble fraction was, however, about twice that for liver microsomes and soluble fraction. Based on the following metabolic scheme the authors speculated that the lung soluble fraction lacked sufficient alcohol dehydrogenase or aldehyde oxidase or both.

$$\text{p-xylene} \xrightarrow[\text{oxidation}]{\text{microsomal}} \begin{array}{l}\text{p-methylbenzyl}\\ \text{alcohol}\end{array} \xrightarrow[\text{dehydrogenase}]{\text{alcohol}} \text{p-methyl benzaldehyde}$$

$$\begin{array}{l}\text{p-methyl benzaldehyde}\\ \text{(p-tolualdehyde)}\end{array} \xrightarrow[\text{oxidase}]{\text{aldehyde}} \begin{array}{l}\text{p-methylbenzoic acid}\\ \text{(toluic acid)}\end{array}$$

This was not entirely the case since lung microsomes incubated with p-xylene in the presence of these two enzymes showed little increase in metabolic rate to toluic acid (Carlone and Fouts, 1974).

Smith et al. (1982) used whole, perfused livers and lungs of rabbits to study the metabolism of p-xylene. The major hepatic metabolite was n-p-toluylglycine (p-methylhippuric acid), which represented 69% of the perfused dose. Unconjugated p-toluic acid represented about 4.5% and p-methylbenzyl alcohol represented ~0.02% of the perfused p-xylene (~20% of the perfused p-xylene was lost through evaporation). Perfusion of p-xylene into the isolated lungs resulted in 32.1, 6.2 and 0.9% of the dose as p-methylbenzyl alcohol, p-toluic acid and 2,5-dimethylphenol, respectively. No glycine conjugate of toluic acid was recovered from the lung. About 15% of the perfused p-xylene was recovered unchanged in the perfusate and ~46% was exhaled from the lung. These data are consistent with those reported by

Carlone and Fouts (1974), indicating that lung tissue is only reactive to microsomal oxidation. The initial oxidation of p-xylene proceeded more rapidly in the lung (328 nmol/min/g tissue) than liver (73 nmol/min/g tissue), but overall the liver had a greater capacity for p-xylene removal from the circulation and subsequent metabolism.

In vitro, with rat liver enzymes, the Michaelis' constants (K_m) for conjugation of m-, o- and p-toluic acid with glycine to form their respective methylhippuric acids were 3.7×10^{-4} M, 2.7×10^{-3} M and 5.2×10^{-4} M (Mikulski et al., 1979b). These data indicate the decreased affinity of the rat liver enzyme for o-toluic acid, which may contribute to o-toluic acid being conjugated alternately with glutathione.

Metabolism of xylenes in humans may be different from that of laboratory animals. Noting the apparent difference among reports in the literature, Ogata et al. (1979) compared the metabolism of o-xylene in female rats with that in human volunteers. Consistent with the studies previously reviewed, female Wistar rats treated with 1.2 g o-xylene/kg (intraperitoneally) excreted predominantly o-toluic and glucuronide (56.6%), and less o-methylhippuric acid (15.9%). In humans ingesting 39 or 78 mg/kg of o-xylene (single oral dose), the major urinary metabolite was o-methylhippuric acid (33-36%), and o-toluic acid glucuronide represented ~1.3% of the administered dose. In volunteers exposed by inhalation (138 ppm o-xylene for 3 hours), similar proportions of metabolites were recovered (Ogata et al., 1980).

In humans, m-xylene is predominantly metabolized to m-methylhippuric acid (Riihimaki et al., 1979a,b). The urinary excretion of metabolites of m-xylene was studied in male volunteers exposed by inhalation. Although several exposure schedules were used by these authors (see Absorption Section for details), excretion of urinary metabolites was studied only after the first day's exposure (~100 ppm for 6 hours). m-Methylhippuric acid accounted for ~84% of the absorbed m-xylene within 24 hours postexposure. 2,4-Xylenol (2,4-dimethylphenol) accounted for 1-2% and excretion of m-xylene in the expired air accounted for ~4%. An additional 16.7-21.3% of the administered dose was collected as urinary m-methylhippuric acid during days 2-6 postexposure.

In several experiments using 6 male volunteers (aged 28-50), the purified xylene isomers were administered separately at 200 and 400 mg/m^3 for 8 hours/day and urinary metabolites were measured during exposure and for 40 hours postexposure (Sedivec and Flek, 1976). No free toluic acid metabolites were discovered in the urine. Conjugated urinary metabolites were restricted to toluic acids bound to glycine (methylhippuric acids) for all isomers. Based on data on rabbits (Bray et al., 1949), Sedivec and Flek (1976) suggested that at high-dose levels of xylene, glycine may be depleted and glucuronide conjugation could occur as an "emergency" (substrate saturation) mechanism. Minor phenolic metabolites identified in the urine after administration of o-, m- or p-xylene were 2,3- and 3,5-xylenol, 2,4-xylenol or 2,5-xylenol, respectively.

Because of the relatively complete and consistent metabolism of xylenes to methylhippuric acids, urinary methylhippuric acid has been used as a measure of occupational exposure to xylenes (Engstrom et al., 1976; Ogata and Hobara, 1979; Sugihara, 1979). Urinary methylhippuric acid was excreted at a level proportional to xylene exposure. Because of the relatively low occurrence of methylhippuric acid in unexposed humans, this parameter may be used to estimate xylene exposure. Caution must be used, however, since o-xylene is apparently metabolized to this conjugate at a proportion relative to dose different from m- or p-xylene.

Excretion

Excretion of the xylenes in laboratory animals and humans is predominantly by urinary elimination of metabolites, but pulmonary exhalation of unchanged solvent does account for ~5% of the absorbed dose (Sedivec and Flek, 1976; Astrand et al., 1978). As reviewed in the Metabolism Section, in animals and humans the major urinary metabolites (80-90% of administered dose) of m- and p-xylene are the corresponding glycine conjugates of toluic acid. In animals, o-xylene is metabolized in nearly equal amounts to o-toluyl glucuronide and o-methylhippuric acid, while urinary excretion of the glucuronide conjugate represents a minor fraction (1-2%) of administered o- and m-xylene isomers. In humans, all isomers appear to be metabolized similarly and excreted almost entirely as methylhippuric acid (Sedivec and Flek, 1976).

Minor metabolites identified in the urine of animals treated with the xylene isomers included mercapturic acid, unconjugated toluic acid and dimethylphenol. o-Xylene may be more readily metabolized to its thio-ester conjugate (o-methylbenzylmercapturic acid) than the m- or p-isomer. The production of various isomers of dimethylphenol by aromatic ring hydroxylation occurs only to a small degree, representing 1-4% of the administered dose in animals or humans. Mercapturic acid has been reported as a minor metabolite in rabbits treated with all of the xylene isomers (Bray et al., 1949, 1950).

The rate of excretion of o-, m- and p-methylhippuric acid was studied in men exposed at ~400 mg/m³ for 8 hours. Urine samples were taken every 2 hours for 14 hours (including exposure period) and at 8-hour intervals for up to 48 hours after the start of exposure. Excretion of the methylhippuric acid metabolites rose quite rapidly during exposure, reaching a mean level of ~135 mg during the first 2 hours of exposure and maximum level of ~260 mg during the final 2 hours of exposure. After exposure was terminated, excretion decreased very rapidly to <50 mg for 4-6 hours postexposure. Throughout the remaining postexposure interval, low levels of methylhippuric acid continued to be excreted. To confirm the speculation that exposure to technical xylene would be similar, Sedivec and Flek (1976) also exposed volunteers to a 1:1:1 mixture of the xylene isomers. Overall, the rate of excretion followed a pattern similar to that seen with the purified isomers. When urinary methylhippuric acids were identified as to isomer, it was apparent that during the first 2 hours of exposure p-methylhippuric acid predominated (41.5%), and o-methylhippuric acid was less (23.8%) than that expected. In later urine samples, each isomeric component approached the expected 33% of the total methylhippuric acid content.

The rate of urinary excretion of m-xylene metabolites in humans was studied in male and female volunteers exposed by inhalation at 100, 300 or 600 mg/m³ (23, 69 or 138 ppm) for 8 hours (Se'nczuk and Orlowski, 1978).

During the first 4 hours of exposure, the rate of urinary m-methylhippuric acid excretion increased rapidly (to ~140 mg/hour for men exposed at 300 mg/m³). Excretion of m-methylhippuric acid continued to increase, though less dramatically, from 4 hours until exposure was terminated (maximum excretion rate of ~160 mg/hour for men exposed at 300 mg/m³). The maximum rates of m-methylhippuric acid excretion in men exposed at 100 and 600 mg/m³ were ~75 and 350 mg/hour, respectively. Maximum excretion rates were reached by the end of the exposure period. After exposure was terminated the rate of m-methylhippuric acid excretion decreased rapidly to near zero by 30 hours. The rate of excretion during the exposure period was dependent on the absorbed dose of m-xylene. Based on these excretion studies, Se'nczuk and Orlowski (1978) suggested that m-xylene does not accumulate in the body (or, if so, to a very small extent) on a day-to-day basis after 8 hours of exposure at the levels tested.

The kinetics of excretion of urinary m-xylene metabolites were studied by Riihimaki et al. (1979a) in a group of six male volunteers exposed by inhalation at 414 mg/m³ for 4.5 days (6 hours/day) followed by a 3-hour exposure at 870 mg/m³ on the fifth day (See details of exposure in Absorption section). The rate of methylhippuric acid excretion rose rapidly during exposure, dropping slightly during a 1-hour lunch break, and reaching a maximum at ~1.5 mmoles/hour (equivalent to ~160 mg m-xylene/hour) within 2 hours postexposure. Elimination following exposure was characterized by two phases, the first rapid rate having a half-time of 1-2 hours and the second halved in ~10 hours. A third, slowest elimination phase was evident after the fifth day of exposure and was considered indicative of mobilization and elimination of cumulated xylene from the tissues. The half-time for this phase was ~20 hours. This third phase of elimination was more pronounced when exercise coincided with exposure and m-xylene uptake was elevated. Cumulation of m-xylene, therefore, appeared greater (Riihimaki et al., 1979b). The rate of pulmonary elimination of m-xylene was described as a two-phase process. The first phase was rapid, with half-times of 0.5-1.0 hours, and the second slower, near 20 hours (Riihimaki et al., 1979a,b).

In male volunteers exposed by inhalation to 870 mg/m³ during rest and exercise at 50 W (series I) or at 435 mg/m³ during rest and exercise at 50 W, 100 W and 150 W (series II) for 2 hours, pulmonary elimination of unchanged xylene (xylene isomers and ethylbenzene) was greatest immediately after exposure (for details of experimental design, see Absorption Section) (Astrand et al., 1978). Subjects exposed in series I (mean total absorbed xylene, 1379 mg) expired 51, 16 and 3 mg xylene, respectively, by 4, 19 and 48 hours postexposure. In series II (mean total absorbed xylene 991 mg), subjects expired 29, 10 and 2 mg, respectively, by 4, 19 and 48 hours after the 2 hours of inhalation exposure. Total pulmonary elimination accounted for 5 and 4% of the absorbed solvent in series I and II, respectively.

Riihimaki and Pfaffli (1978) reported that elimination of m-xylene in the exhaled air following percutaneous exposure (3.5 hours) to vapor at 600 ppm (~44.5 mg m-xylene absorbed) was ~8 mg/m³ during the exposure period. Pulmonary excretion rate following inhalatory exposure at 20 ppm for 3.5 hours (~37.9 mg m-xylene absorbed) was comparable. The kinetics of urinary excretion of m-methylhippuric acid following percutaneous (600 ppm) or inhalation (20 ppm) exposure was similar to that noted by Se'nczuk and Orlowski (1978). Initially, excretion is very rapid so that nearly 50% of the absorbed dose is excreted within the 35-hour exposure period. About 80%

of the absorbed dose is excreted within 4 hours postexposure. After this period the excretion rate slows somewhat but appears complete within ~24 hours (Riihimaki and Pfaffli, 1978). The maximum excretion rate of m-methylhippuric acid in volunteers who immersed both hands in pure m-xylene for 15 minutes (~22.6 mg m-xylene absorbed) was ~5.3 mg/hour, occurring immediately after exposure was terminated (Riihimaki, 1979a).

Riihimaki (1979b) suggested that the conjugation of methylbenzoic acid with glycine forming methylhippuric acid was the rate-limiting step in xylene metabolism and elimination. This suggestion was based on excretion kinetics data obtained from one male volunteer treated orally and intravenously with methylbenzoic acid and methylhippuric acid, and also by analogy with toluene excretion. The rate of excretion of methylhippuric acid was notably slower when methylbenzoic acid was administered, necessitating glycine conjugation. The maximum rate of excretion approximated the maximum capacity of glycine conjugation (190 μmole/minute). Riihimaki (1979b) further speculated that occupational exposure to xylene vapor at 780 ppm (3385 mg/m³) during light work or 270 ppm (1172 mg/m³) during moderate work for 8 hours/day (assuming 60% pulmonary absorption) would saturate the capacity of xylene metabolism. Possible hazardous accumulation of xylene or its metabolites may occur thereafter.

In an effort to determine a reliable method for estimating occupational xylene exposure, Engstroem et al. (1980) measured exposure levels of xylene and urinary methylhippuric acid, exhaled air and blood xylene levels of 15 painters. Breathing zone samples were taken on 3 days (Wednesday-Friday) to determine daily TWA xylene levels. Following the exposure day, xylene levels in the blood and expired air and urinary methylhippuric acid were measured at various postexposure times and correlated to the TWA. TWA xylene levels were as high as 70 ppm. Exhaled air (r=0.63) and blood (r=0.75) xylene levels correlated poorly with different xylene exposures, while urinary methylhippuric acid (r=0.92) correlated quite well. Exposure to ~50 ppm xylene (TWA) resulted in urinary methylhippuric acid levels of ~500 mg/g creatinine during the last 4-5 hours of the daily exposure. Engstroem et al. (1980) suggested urinary methylhippuric acid could give a good estimate of occupational TWAs of xylene.

Summary

Data on the pharmacokinetics of xylenes following oral exposure are limited. Inhalation and dermal exposures to this ubiquitous solvent have been more industrially important and, therefore, more completely studied.

Based on inference from limited excretion data, absorption of xylenes from the gastrointestinal tract appears complete. Pulmonary absorption based on several studies is ~60% efficient. Absorption of xylene vapor through the skin is much slower than pulmonary absorption and appears to represent 1-2% of that absorbed through the lungs (Riihimaki and Pfaffli, 1978). Absorption as high as 9.6 mg/cm^2/hour for pure xylene applied to the forearm was reported (Dutkiewicz and Tyras, 1968).

Distribution of xylene throughout the body appears rapid (Carlsson, 1981). Bioaccumulation may occur during high-level or extended xylene exposure in the adipose tissue, but most xylene is cleared rapidly from the body as it is metabolized. The half-life of m-xylene in the adipose tissue following exposure at 100 ppm (6 hours/day for 6 days) was reportedly 47.5 hours (Engstrom and Riihimaki, 1979). Savolainen et al. (1979) reported that accumulation of xylene in the perirenal fat of rats exposed by inhalation (300 ppm for 6 hours/day, 5 days/week for periods of 5-18 weeks) was initially high (after 5 weeks of exposure), but decreased with longer exposure durations. The gradual decrease was correlated with increased xylene metabolism during longer exposure periods.

In animals and humans, xylene isomers are predominantly (85-95% of absorbed dose) metabolized by oxidation of one of the methyl groups. The resultant product is toluic acid, which is conjugated for urinary excretion. Evidence from animal and human studies indicates that both m- and p-toluic acid metabolites are conjugated with glycine forming m- or p-methylhippuric acid, respectively. In animals, o-toluic acid conjugates equally well with glucuronide, resulting in both o-methylhippuric and o-toluyl glucuronide in the urine. In humans, o-toluic acid appears to be conjugated primarily with glycine so that o-methylhippuric acid predominates. Aromatic hydroxylation of all xylene isomers represents a minor (1-2%) metabolic pathway in animals and humans.

Elimination of xylenes is primarily through urinary excretion of metabolites, which may represent nearly 95% of the absorbed dose. Exhalation of unchanged xylene from the lungs may represent 5% of the absorbed dose. In humans exposed to m-xylene by inhalation at 600 mg/m^3 (138 ppm) for 8 hours, the maximal urinary excretion rate was 350 mg/hour. Excretion rate was related to the absorbed dose and did not appear to be saturated at this

IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as xylene from a variety of sources, including drinking water, food, ambient air, occupational settings and consumer products. This analysis of human exposure to xylene is limited to drinking water, food and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works and travels, what one eats, and physiologic characteristics related to age, sex and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

In the Exposure Estimation Section of this chapter available information is presented on the range of human exposure and intake for xylene from drinking water, food and ambient air for the 70 kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combined exposures from those three sources. However, the Summary Section of this chapter provides some insight into the relative contributions of the three sources, especially drinking water, to the range of intake values suggested by the available data.

Exposure Estimation

Drinking Water. Data on the occurrence of xylene in public drinking water supplies are available from four Federal surveys [the National

Organics Reconnaissance Survey (NORS), the 1978 Community Water Supply Survey (CWSS), the Groundwater Supply Survey (GWSS) and the National Screening Program for Organics in Drinking Water (NSP)] and from regional and state data.

In the NORS (U.S. EPA, 1975a), analyses for organic contaminants in the finished drinking water of 10 cities were conducted. Xylene was not detected in two cities using a groundwater source. Xylene was observed in one of the eight cities using surface water as a source; it was reported only as "detected" in this city. A detection limit was not given.

The CWSS (U.S. EPA, 1978) examined 106 surface water supplies, 330 groundwater supplies and 16 supplies with mixed water or purchased water sources in the United States in 1978. Samples were analyzed for various volatile organic chemicals, including xylene. One to five samples, including raw, finished and/or distribution water, were collected from each system. Data on xylene (undifferentiated by isomer) in finished or distribution water were available for 289 of the groundwater supplies and 97 of the surface water supplies sampled. Less than 3% of the sampled groundwater systems contained detectable levels of xylene [minimum quantitation limit (MQL) = 0.5 μg/ℓ]. Xylene was found in seven groundwater systems with a mean concentration of 1.06 μg/ℓ (range = 0.6-2.5 μg/ℓ). Six surface water supplies (6%) were positive with a mean concentration of 1.66 μg/ℓ (range = 0.635-5.2 μg/ℓ). [Ninety surface water systems were negative with the MQL at 0.5 μg/ℓ; 1 system was negative with the MQL at 0.625 μg/ℓ.]

The GWSS (U.S. EPA, 1983) examined a national sampling between December 1980 and December 1981 of 945 public water supply systems using groundwater sources for over 30 organic chemicals. In the GWSS there were 456 randomly selected public water supplies and 473 nonrandomly selected public water supplies, selected with state and U.S. EPA regional input based on the likelihood of finding contamination. For both samplings data was presented for m-xylene, and the combined ortho- and para-xylene isomers. The MQL for the xylenes was 0.2 μg/ℓ. m-Xylene and o/p-xylene were both detected in eight (1.8%) of the randomly selected supplies, with mean concentrations of 0.55 μg/ℓ (range = 0.20-1.5 μg/ℓ) and 0.43 μg/ℓ (range = 0.26-0.91 μg/ℓ), respectively. In the nonrandom supply sampling, m-xylene was detected in eight supplies (1.7%) and o/p-xylene was detected in 10 supplies (2.1%), with mean concentrations of 0.44 μg/ℓ (range = 0.24-0.83 μg/ℓ) and 0.71 μg/ℓ (range = 0.27-2.5 μg/ℓ), respectively. [Note: Seven of the nonrandom supplies had both m-xylene and o/p-xylene.]

The NSP, conducted by SRI International (Boland, 1981) from June 1977 to March 1981, examined raw and finished water samples from water facilities in 33 states. Samples were analyzed for many halocarbons, pesticides and other chemicals (including xylene). Finished water samples from 99 surface water supplies and 11 groundwater supplies were analyzed for o-, m- and p-xylene. The compounds were not detected in any of the supplies (MQL = 0.3 μg/ℓ).

The U.S. EPA (1984) provided information from a Region VII survey. During the period from June 1982 to August 1982, 272 sites in the states of Kansas, Missouri and Nebraska were sampled. Data were presented for m-xylene and the combined isomers of o/p-xylene (MQL = 0.2 μg/ℓ).

m-Xylene and o/p-xylene were both found in eight supplies, with median concentrations of 1.2 µg/ℓ (range = 0.2-18 µg/ℓ) and 1.9 µg/ℓ (range = 0.2-44 µg/ℓ), respectively.

o-Xylene was observed in the New Orleans Area Water Supply Study conducted in 1974 (U.S. EPA, 1975b). o-Xylene was found in all three water plants sampled in the New Orleans study, with maximum measured concentrations of 2.8, 3.4 and 4.1 µg/ℓ.

In a study by the state of New York, conducted between September 1980 and January 1981, samples of water from 57 groundwater supplies, 9 surface water supplies and 3 mixed source supplies were analyzed for all three isomers of xylene. No isomer of xylene was detected in any of the samples taken (detection limit = 1 µg/ℓ) (Close et al., 1982).

Additional studies in New York and Indiana also provided information to EPA on the occurrence of xylene in state drinking water supplies. Three samples of finished groundwater from Waterford, New York contained xylene (unspecified isomers) with a range from 0.94-1.0 (NY-01). Xylene (unspecified isomers) was detected in three samples of finished groundwater from Elkhart, Indiana (range = 41-130 µg/ℓ) (IN-01). m-Xylene was detected in four groundwater samples from Elkhart, Indiana (water type unspecified) with a range from 0.6-630 µg/ℓ (IN-01). The combined isomers of o/pxylene were also reported as being detected in four groundwater samples (tap water) from Elkhart, Indiana with a range from 20-750 µg/ℓ (IN-01).

Assuming that m-xylene is present at the highest concentration reported, i.e., 630 μg/ℓ in Elkhart, the daily intake for the 70 kg adult male consuming 2 ℓ/day of drinking water would be 1260 μg/day or 18 μg/kg/day.

Assuming that o/p-xylene is present at the highest concentration reported, i.e., 750 μg/ℓ in Elkhart, the daily intake for the 70 kg adult male consuming 2 ℓ/day of drinking water would be 1500 μg/day or 21.4 μg/kg/day. Therefore, the maximum daily intake for total xylene would be 2760 μg/day or 39.4 μg/kg/day.

Diet. No information was obtained on the occurrence of xylenes in food. Consequently, dietary intake and the contribution of dietary intake to total exposure could not be evaluated.

Air. Brodzinsky and Singh (1982) have compiled available published and unpublished atmospheric data on volatile organic chemicals, including o-xylene and m/p-xylene, into a master file, and have subjected these data to statistical analysis. An assessment of the quality, reliability and representativeness of the data was also performed. Each data point was given a "quality code" based on either a quantitative assessment or on the authors' own experience and judgment. Quality codes were: 1 = Excellent; 2 = Good; 3 = Acceptable; 4 = Questionable; and Z = Unable to assign quality.

Using data for quality codes 1-3, Brodzinsky and Singh (1982) calculated the following median concentrations of o-xylene: rural/remote areas, 0.41 μg/m³ (114 observations); urban/suburban areas, 5.2 μg/m³ (1885 observations); and source dominated areas, 3.5 μg/m³ (183 observations).

Median concentrations for m/p-xylene are as follows: rural/remote areas, 0.38 µg/m³ (115 observations); urban/suburban areas, 12 µg/m³ (1911 observations); and source dominated areas, 7.4 µg/m³ (186 observations). The highest levels for o-xylene and m/p-xylene measured, both of which were from a source dominated location, were 38,000 and 43,000 µg/m³, respectively (Oldham et al., 1979).

The daily respiratory intake of o-xylene and m/p-xylene from air were estimated using the assumptions presented in Tables IV-1 and IV-2 and the median and maximum levels for the xylenes reported above. The estimates in Table IV-1 indicate that the daily o-xylene intake from air for adults exposed to the median levels in source dominated areas is approximately 1 µg/kg/day. In contrast, the intake calculated using the maximum level reported is 12,000 µg/kg/day; few if any persons are believed to be exposed at that level.

Similarly, the estimates in Table IV-2 indicate that the daily m/p-xylene intake from air for adults exposed to the median levels in source dominated areas is ~2 µg/kg/day. In contrast, the intake calculated using the maximum level reported is 14,000 µg/kg/day; again, few if any persons are believed to be exposed at that level. The values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

TABLE IV-1

Estimated Respiratory Intake of o-Xylene

Location	Exposure ($\mu g/m^3$)	Intake	
		($\mu g/kg$)	($\mu g/kg/day$)
Rural/remote	0.41	9.4	0.1
Urban/suburban	5.2	119.6	1.7
Source dominated	3.5	80.5	1.2
Maximum	38,000	870,000	12,000

Assumptions: 70 kg man, 23 m^3 of air inhaled/day (ICRP, 1975)

TABLE IV-2

Estimated Respiratory Intake of m/p-Xylene

Location	Exposure (μg/m³)	Intake (μg/kg)	Intake (μg/kg/day)
Rural/remote	0.38	8.7	0.1
Urban/suburban	12.0	276.0	3.9
Source dominated	7.4	170.2	2.4
Maximum	43,000	990,000	14,000

Assumptions: 70 kg man, 23 m³ of air inhaled/day (ICRP, 1975)

Summary

Table IV-3 presents a general view of the total amount of xylene received by a 70 kg adult male from air, food and drinking water. Three separate exposure levels in air, five exposure levels in drinking water, and one exposure level from foods are shown in the table. Intakes have been estimated for total xylene (combined isomers).

The data presented have been selected from an infinite number of possible combinations of concentrations for the three sources. The actual exposures encountered would represent some finite subset of this infinite series of combinations. Whether exposure occurs at any specific combination of levels is not known; nor is it possible to determine the number of persons that would be exposed to xylene at any of the combined exposure levels. The data presented represent possible exposures based on the occurrence data and the estimated intakes.

The drinking water levels selected are representative of the range of values observed in the various Federal survey and state data. It is assumed that the 70 kg adult male consumes 2 ℓ/day of drinking water.

Because of the lack of information available on the presence of xylene in food, it is assumed that food does not contribute to the daily intake of xylene.

TABLE IV-3

Estimated Intake of Xylene from the Environment by Adult Males in µg/kg/day

Concentration in Drinking Water (µg/ℓ)	Estimated Total Intake in µg/kg/day (% from drinking water) Based on Air Levels of: Rural/Remote (0.79 µg/m³)	Urban/Suburban (17.2 µg/m³)	Maximum (81,000 µg/m³)
0.0	0.26 (0%)	5.7 (0%)	27,000 (0%)
0.5	0.27 (5.2%)	5.7 (0.24%)	27,000 (0.00005%)
5.0	0.40 (35%)	5.8 (2.4%)	27,000 (0.0005%)
10.0	0.55 (53%)	6.0 (4.8%)	27,000 (0.001%)
1500	43 (100%)	49 (88%)	27,000 (0.16%)

Intake from each source:

Water:
0.5 µg/ℓ: 0.014 µg/kg/day
5.0 µg/ℓ: 0.14 µg/kg/day
10.0 µg/ℓ: 0.29 µg/kg/day
1500 µg/ℓ: 43 µg/kg/day

Air*:
0.79 µg/ℓ: 0.26 µg/kg/day
17.2 µg/ℓ: 5.7 µg/kg/day
81,000 µg/ℓ: 27,000 µg/kg/day

Food: 0 µg/kg/day

*Values are total values for m- and o/p-xylene.

Brodzinsky and Singh (1982) calculated a median urban/suburban air level for o-xylene of 5.2 µg/m³ and for m/p-xylene of 12 µg/m³ based on air monitoring data. Assuming a total (combined isomers) air level of 17.2 µg/m³ and the estimated xylene intake of 0 µg/kg/day from foods, drinking water would be the predominant source of xylene exposure in the adult male only at drinking water levels >200 µg/ℓ. An accurate assessment of the number of individuals for which drinking water is the predominant source of exposure cannot be determined from the data since specific locations containing high concentrations of xylene in drinking water and low concentrations of xylene in ambient air and food are unknown.

The relative source contribution data are based on estimated intake and do not account for a possible differential absorption rate for xylene by route of exposure. The relative dose received may vary from the relative intake. In addition, the relative effects of the chemical on the body may vary by different routes of exposure.

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V. HEALTH EFFECTS IN ANIMALS

Based on reviews of the early literature on the toxicity of xylenes to animals, Browning (1965) and the National Academy of Sciences (NAS, 1980) noted the different toxicities elicited by the various isomers. No consistent pattern exists in showing one isomer to be more toxic than the others and each, administered by various routes, has been denoted as more toxic than the others. This review will emphasize recently published data, studies that characterize xylene toxicity following administration by relevant routes and studies that specify and delineate the toxicity of the different xylene isomers.

General Toxicity

Acute Toxicity. Data on the lethality of the xylene isomers following acute exposure by oral, inhalation and dermal routes are summarized in Table V-1. Data on rats predominate, but an early study (Cameron et al., 1938) suggests that mice treated by inhalation are the more susceptible species. A ranking for the xylene isomers by toxic potential is not demonstrated. By oral administration, o-xylene may be slightly more toxic (Gerarde, 1959; Ungvary et al., 1979), but by inhalation m- and p-xylenes have been implicated as being the more toxic isomers (Cameron et al., 1938; Bonnet et al., 1979).

Generally, xylene has a low toxicity, with oral LD_{50} values ranging from 3.6-6.75 g/kg for the isomers and 4.3-6.1 g/kg for xylene (assumed to be a mixture of isomers). Mixed xylene (assumed to contain xylene isomers and ethylbenzene) was reported to have an LD_{50} of 8.8 g/kg (Hine and

TABLE V-1

Acute Lethality of Xylenes

Route of Exposure	Species/Strain	Sex	Number	Compound	Purity	Dose	Parameter/Effect	Reference
Oral	rat/Carworth-Wistar	M	5	m-xylene	NR	6.75 g/kg	LD_{50}	Smyth et al., 1962
Oral	rat/NS	NS	NS	o-xylene	NS	3.6 g/kg	LD_{50}	Ungvary et al., 1979
Oral	rat/NS	NS	NS	m-xylene	NS	5.1 g/kg	LD_{50}	Ungvary et al., 1979
Oral	rat/NS	NS	NS	p-xylene	NS	4.1 g/kg	LD_{50}	Ungvary et al., 1979
Oral	rat/albino	F	NS	xylene	NS	6.1 g/kg	LD_{50}	Muralidhara, 1980
Oral	rat/Long-Evans	M	6	mixed xylene	NR	8.8 g/kg	LD_{50}	Hine and Zuidema, 1970
Oral	rat/Wistar	M	20	xylene	m-xylene 52% p-xylene 24% o-xylene 19%	4.3 g/kg	LD_{50}	Wolf et al., 1956
Oral	rat/NR	NR	10	o-xylene	NR	6.26 g/kg	mortality, 7/10	Gerarde, 1959
Oral	rat/NR	NR	10	m-xylene	NR	6.14 g/kg	mortality, 3/10	Gerarde, 1959
Oral	rat/NR	NR	10	p-xylene	NR	6.12 g/kg	mortality, 6/10	Gerarde, 1959
Oral	rat/NS	NS	NS	xylene	NS	9 g/kg	100% mortality	Faustov, 1960
Inhalation	mice/NR	NR	10	o-xylene	NR	saturated vapor (12,250 ppm) x 12 hours	mortality, 2/10	Cameron et al., 1938
Inhalation	mice/NR	NR	10	o-xylene	NR	6125 ppm x 24 hours	mortality, 9/10	Cameron et al., 1938
Inhalation	mice/NR	NR	10	o-xylene	NR	3062 ppm x 24 hours	mortality, 4/10	Cameron et al., 1938
Inhalation	mice/NR	NR	10	o-xylene	NR	1531 ppm x 24 hours	mortality, 0/10	Cameron et al., 1938
Inhalation	mice/NR	NR	10	m-xylene	NR	saturated vapor (8040 ppm) x 12 hours	mortality, 6/10	Cameron et al., 1938
Inhalation	mice/NR	NR	10	m-xylene	NR	2010 ppm x 24 hours	mortality, 6/10	Cameron et al., 1938
Inhalation	mice/NR	NR	10	m-xylene	NR	1005 ppm x 24 hours	mortality, 0/10	Cameron et al., 1938

TABLE V-1 (cont.)

Route of Exposure	Species/Strain	Sex	Number	Compound	Purity	Dose	Parameter/Effect	Reference
Inhalation	mice/NR	NR	10	p-xylene	NR	saturated vapor (19,650 ppm) x 12 hours	mortality, 9/10	Cameron et al., 1938
Inhalation	mice/NR	NR	10	m-xylene	NR	4912 ppm x 24-28 hours	mortality, 0/10	Cameron et al., 1938
Inhalation	mice/NR	NR	10	m-xylene	NR	2451 ppm x 24 hours	mortality, 0/10	Cameron et al., 1938
Inhalation	rat/albino	M/F	6	m-xylene	NR	concentrated vapor	2 hours maximum exposure for ho deaths	Smyth et al., 1962
Inhalation	rat/albino	M/F	6	m-xylene	NR	8000 ppm x 4 hours	mortality of 10/12	Smyth et al., 1962
Inhalation	rat/Long-Evans	M	10	mixed xylene	NR	6350 ppm x 4 hours	LC_{50}; all deaths occurring during exposure, survivors were briefly comatose	Hine and Zuidema, 1970
Inhalation	rat/Wistar	NR	10	o-xylene	NR	saturated vapor (12,250 ppm) x 12 hours	mortality, 2/10	Cameron et al., 1938
Inhalation	rat/Wistar	NR	10	o-xylene	NR	6125 ppm x 24 hours	mortality, 8/10	Cameron et al., 1938
Inhalation	rat/Wistar	NR	10	o-xylene	NR	3062 ppm x 24 hours	mortality, 1/10	Cameron et al., 1938
Inhalation	rat/Wistar	NR	10	o-xylene	NR	1531 ppm x 24 hours	mortality, 0/10	Cameron et al., 1938
Inhalation	rat/Wistar	NR	10	m-xylene	NR	saturated vapor (8040 ppm) x 12 hours	mortality, 8/10	Cameron et al., 1938
Inhalation	rat/Wistar	NR	10	m-xylene	NR	2010 ppm x 24 hours	mortality, 0/10	Cameron et al., 1938
Inhalation	rat/Wistar	NR	10	m-xylene	NR	1005 ppm x 24 hours	mortality, 0/10	Cameron et al., 1938
Inhalation	rat/Wistar	NR	10	p-xylene	NR	saturated vapor (19,650 ppm) x 12 hours	mortality, 8/10	Cameron et al., 1938
Inhalation	rat/Wistar	NR	10	p-xylene	NR	4912 ppm x 24-48 hours	mortality, 0/10	Cameron et al., 1938
Inhalation	rat/Wistar	NR	10	p-xylene	NR	2451 ppm x 24-48 hours	mortality, 0/10	Cameron et al., 1938

TABLE V-1 (cont.)

Route of Exposure	Species/Strain	Sex	Number	Compound	Purity	Dose	Parameter/Effect	Reference
Inhalation	rat/NR	M	10 each dose	mixed xylene	m-xylene, 65% p-xylene, 7.8% o-xylene, 7.6%	9900, 6000, 2800, 1300 or 580 ppm x 4 hours	mortality, 10/10, 4/10, 0/10, 0/10, 0/10, respectively	Carpenter et al., 1975
					ethylbenzene, 19.3%	6700 ppm x 4 hours	calculated LC_{50}	
Inhalation	rat/NS	NS	NS	xylene	NS	75,000 mg/m^3 (17,250 ppm) x 2 hours	100% mortality	Faustov, 1960
Inhalation	mice/SPF-OF1	F	25	m-xylene	97%	5267 ppm x 6 hours	LC_{50}	Bonnet et al., 1979
Inhalation	mice/SPF-OF1	F	25	o-xylene	98%	4595 ppm x 6 hours	LC_{50}	Bonnet et al., 1979
Inhalation	mice/SPF-OF1	F	25	p-xylene	98%	3907 ppm x 6 hours	LC_{50}	Bonnet et al., 1979
Dermal	rabbit/New Zealand	M	4	m-xylene	NR	12.4 g/kg	LD_{50}	Smyth et al., 1962
Dermal	rabbit/New Zealand	M	3	mixed xylene	NR	4.4 g/kg x 4 hours contact	mortality, 1/3	Hine and Zuidema, 1970

NS = Not specified in available abstract; NR = Not reported

Zuidema, 1970). The LC_{50} of p-, o- and m-xylene vapor in mice exposed for 6 hours were 3907, 4595 and 5267 ppm (16,960, 19,940 and 22,860 mg/m³), respectively (Bonnet et al., 1979). Percutaneous absorption of xylene is significant since an LD_{50} of 12.4 g/kg was reported for rabbits (2.5-3.5 kg) exposed (xylene applied to the entire clipped body and covered by plastic film) to the pure liquid for 24 hours (Smyth et al., 1962).

The effects of acute orally administered o-, m- and p-xylene were studied in male and female CFY rats (Ungvary et al., 1981a). The xylene isomers were given to groups of eight animals at 2.2 g/kg/day for 4 days. Female rats appeared more sensitive with 20% mortality by the fourth day of exposure to any isomer. Generalized diapedetic hemorrhage was noted in several organs. No mortality occurred among males. All isomers decreased body weight gain and increased liver weights in both sexes. Spleen and thymus weights were decreased in most exposed groups and, histologically, the cortex of the thymus became thin. Hypertrophy of the centrilobular hepatocytes was evident upon histological analyses of exposed rat livers. Centrilobular hepatocytes had proliferative smooth endoplasmic reticulum and glycogen depletion upon ultrastructural analyses. These effects were minimal in rats exposed to o-xylene, while those exposed to m- or p-xylene additionally had abnormally shaped mitochondria. Effects of the xylene isomers on hepatic enzyme levels were reported and are discussed in detail in the Enzyme Induction in the Liver Section in Chapter VII.

Male Sprague-Dawley rats (n=12/group) were treated by gastric intubation with o-, m- and p-xylene at 1.1 g/kg/day for 3 days (Pyykko, 1980). A vehicle control group given corn oil was maintained similarly. All rats survived treatment, appeared healthy and behaved with no apparent anesthesia. All animals lost weight because of the schedule of food availability, but the treatment groups lost significantly ($p<0.05$) more weight (6-10% weight reduction). Mean liver weights in animals treated with o- and m-xylene were significantly ($p<0.05$) increased. Kidney weights were slightly elevated, but only significantly so following m-xylene treatment. Lung weights were not affected. Effects on levels of enzymes and cytochromes in these organs are discussed in Chapter VII.

Doses of 1.3 g of o-, m- or p-xylene per rabbit (average body weight not specified) by stomach tube reportedly caused no toxic effects (Bray et al., 1949). Oral doses of 1.75 g of m- or p-xylene per rabbit were also tolerated with no apparent toxic effects. o-Xylene administered at this dose, however, caused the excretion of excessive amounts of "reducing material" (nonmetabolite compound), which was regarded as a manifestation of toxicity (Bray et al., 1949).

The effects of acute exposure to xylene (pure mixture of isomers) by inhalation were reported for male Long-Evans rats (100-200 g bw) exposed for 2 hours (Furnas and Hine, 1958). Exposure at 4000 ppm (17,360 mg/m^3) for 2 hours caused no apparent effects. Xylene at 20,000 ppm (86,800 mg/m^3; approximately saturated vapor) for 2 hours caused effects comparable with those of benzene (at 10,000 ppm), which included difficulty in breathing and neuromuscular disorders (ataxia, muscular twitching, muscular spasms and general body quivering). Similar effects were evident among 12 rats exposed for four exposures of 50, 45, 18 and 40 minutes duration, respectively, at 10,000 ppm (43,400 mg/m^3). Five of the animals died as a result of this exposure. The interval between exposures was not explicated, but rats were removed from exposure when convulsions or respiratory arrest was apparent and returned "as soon as they recovered." Upon necropsy, no lesions were identified microscopically (emphasis was placed on neurohistology), but the respiratory tract showed evidence of local irritation (Furnas and Hine, 1958).

Prostration was the first effect reported in rats exposed to mixed xylene (65% m-xylene, 7.8% p-xylene, 7.6% o-xylene and 19.3% ethylbenzene) at various levels for up to 4 hours (Carpenter et al., 1975). At 26,000 mg/m³ (6000 ppm), rats became prostrate within 30 minutes and 4/15 died before exposure was terminated. All rats (n=15) exposed to 43,000 mg/m³ (9900 ppm) died within 135 minutes. At 12,000 mg/m³ (2800 ppm), pulmonary and conjunctival irritation was evident and rats were prostrate by 2-3.5 hours but recovered after exposure. At 5800 mg/m³ (1300 ppm) for up to 4 hours, rats showed signs of poor coordination but not prostration. A level of 2500 mg mixed xylene/m³ (580 ppm) for a 4-hour exposure caused no signs of distress or toxicity among the exposed rats. After examination of the rats that died after exposure at 43,000 mg/m³, six cases with treatment-related lung pathologies (2 cases each of edema, hemorrhage and atelectosis) were reported. In another group of five rats exposed at 46,000 mg/m³ (11,000 ppm) for up to 2 hours, eye and extremity irritation was evident within 5 minutes, with prostration within 20 minutes, neuromuscular tremors within 45 minutes, two deaths within 66 minutes and two more deaths by 80 minutes.

In mice exposed to the mixed xylenes (same composition as that used in rats) for only 1 minute, a 50% depression of the respiratory rate was noted when exposure was at ~5600 mg/m³ (1300 ppm) (Carpenter et al., 1975). Respiratory rate was decreased by 50% in all mice exposed at 53,000 mg/m³ (12,000 ppm) and in 4/6, 2/6 and 2/6 of those exposed at 28,000, 11,000 and 5600 mg/m³, respectively. Exposure at 2000 mg/m³ (460 ppm) for 1 minute had no effect on respiratory rate.

In an early toxicity study, Batchelor (1927) treated white rats with xylenol (xylene) by intraperitoneal and subcutaneous injection and by inhalation. Single intraperitoneal injections of up to 650 mg/kg (in olive oil) caused no neurological symptoms. Narcosis was produced at higher dose levels, and the lethal dose was reportedly ~2.15 g/kg. Subcutaneous administration of ~0.86-1.75 g xylene/kg (in olive oil) for 10 consecutive days produced only minor effects on activity or weight. Microscopic examinations revealed bone marrow hyperplasia, splenic hyperplasia, hepatic centrilobular necrosis and renal cloudy swelling. These effects, especially the first two, may have been caused by benzene contamination of the xylene used in this study (purity not specified). Inhalation studies were conducted using xylene levels ranging from 620-1600 ppm (2697-6960 μg/m³); daily exposure durations ranged from 18-120 hours and at 1600 ppm (6960 μg/m³) rats were unstable, incoordinate and developed narcosis that eventually became immobilizing. At 980 ppm, instability and incoordination developed, but narcosis was not produced. Levels of 620 ppm were regarded as being "slight, if at all, toxic" (Batchelor, 1927).

The effects of intraperitoneally injected xylene (analytical purity) on behavioral activity was studied in male CFY rats (Paksy et al., 1982). Approximate dose levels were 0.32, 0.39, 0.60, 0.90 and 1.50 g/kg in sunflower oil; behavioral tests performed were open-field behavior, wheel running, tilted plane and inversion performance. Xylene caused significant ($p<0.01$) decreases in open-field ambulation and inversion performance at doses $\geq$0.90 g/kg; rearing activity and tilted plane performance was inhibited at $\geq$0.60 g/kg. Oddly, wheel running activity was not significantly different from controls in rats exposed to xylene at any dose level tested.

Following the application of undiluted xylene to the ears and shaved abdomen of white rabbits (10-20 applications applied over 2-4 weeks), Wolf et al. (1956) reported moderate to marked irritation with erythema and moderate superficial necrosis ("chapped" appearance with exfoliation). Conjunctival irritation and corneal injury were determined in rabbits having two drops of undiluted xylene placed in the right eye. Visual observations of the treated eye were made at 3 minutes, 1 hour, and 1, 2 and 7 days postexposure. Corneal injury was determined by fluorescein staining at 3 minutes postexposure. Irritation of the conjunctival membranes and very slight superficial necrosis of the cornea was reported (Wolf et al., 1956). Similarly, Hine and Zuidema (1970) reported skin irritation on intact and abraded areas when xylene was applied to shaved rabbits. Applied xylene was covered with gauze and, at most, four applications were made every 24 hours. Conjunctival irritation was also reported in all six rabbits treated with 0.1 μℓ xylene placed in the eye. Irritation was greatest at 48 hours postexposure. Smyth et al. (1962) also reported moderate irritation to rabbit skin (n=5) when xylene (0.01 mℓ) was applied, uncovered and checked after 24 hours. Only small areas of corneal necrosis resulted when 0.5 mℓ of undiluted xylene was applied into the eyes of rabbits.

Subchronic and Chronic Toxicity. o-Xylene was administered to aging male Long-Evans rats (12-19 months old) in the diet at 200 mg/kg of feed for up to 6 months (Bowers et al., 1982) (Table V-2). Twenty rats weighing 0.8-0.9 kg were used in each group (exposed and control) and five animals of each group were killed after 1, 2, 3 and 6 months of exposure. Livers of sacrificed animals were fixed for electron microscopy by perfusion. Upon gross examination, the livers of all treated rats exposed for any interval tested appeared normal. Ultrastructurally, hepatocytes from rats exposed for 1, 2, 3 or 6 months had two types of vesicles not present in hepatocytes of control animals. These structures were apparent after 1 month of exposure but did not increase in number after longer exposures. One vesicle was double membrane-bound and appeared to originate from the smooth endoplasmic reticulum. The second type of vesicle was peripherally located and appeared to fuse to the plasmalemma as in exocytosis. The first vesicle may have been of an autophagic type derived from the smooth endoplasmic reticulum and involved in elimination of the xylene. The second type was considered to be involved with increasing hepatocyte surface area or the elimination of metabolites.

No visible effects or deaths were reported among mice or rats exposed to p-, m- or o-xylene at low levels by inhalation for 14 exposures (Table V-2) (Cameron et al., 1938). Ten mice and 10 rats were exposed to p-xylene vapor at 5300 mg/m³ (1226 ppm) for 14 daily 8-hour exposures. Two additional

TABLE V-2

Subchronic Toxicity of Xylenes in Laboratory Animals

Route	Species/Strain	Sex	Number	Compound	Exposure Level	Duration	Effect	Reference
Oral	rat/Long-Evans	M	20	o-xylene	200 mg/kg diet	6 months	ultrastructural hepatocyte changes	Bowers et al., 1982
Inhalation	mouse/NR	M/F	10	p-xylene	5300 mg/m³	8 hours x 14 exposures	no mortality; no toxic effects	Cameron et al., 1938
Inhalation	rat/Wistar	M/F	10	p-xylene	5300 mg/m³	8 hours x 14 exposures	no mortality; no toxic effects	Cameron et al., 1938
Inhalation	rat/Wistar	M/F	10	m-xylene	4300 mg/m³	8 hours x 14 exposures	no mortality; no toxic effects	Cameron et al., 1938
Inhalation	rat/Wistar	M/F	10	o-xylene	7300 mg/m³	8 hours x 14 exposures	no mortality; no toxic effects	Cameron et al., 1938
Inhalation	rat/Wistar	M	15	m-xylene	212 mg/m³	6 hours/day, 5 days/week x 2 weeks	decreased brain glutathione, increased brain NADPH-diaphorase	Savolainen and Pfaffli,. 1980
Inhalation	rat/Wistar	M	15	m-xylene	1710 mg/m³	6 hours/day, 5 days/week x 2 weeks	decreased brain glutathione and superoxide dismutase; increased NADPH-diaphorase and azoreductase	Savolainen and Pfaffli, 1980
Inhalation	rat/Wistar	M	15	m-xylene	3220 mg/m³	6 hours/day, 5 days/week x 2 weeks	decreased brain glutathione and superoxide dismutase; increased NADPH-diaphorase and azoreductase	Savolainen and Pfaffli, 1980
Inhalation	rat/CFY	M	30	o-xylene	3500 ppm (15,190 mg/m³)	8 hours/day x 6 weeks	decreased body weight, hepatomegaly, ultrastructural hepatocyte changes	Tatrai and Ungvary, 1980
Inhalation	rat/Sprague-Dawley or Long-Evans	M/F	12	o-xylene	3358 mg/m³	8 hours/day, 5 days/week x 6 weeks	weight gain and hematology not affected; 3 deaths	Jenkins et al., 1970
Inhalation	rat/Sprague-Dawley or Long-Evans	M/F	14	o-xylene	337 mg/m³	continuous for 90 days	weight gain and hematology not affected; 1 death	Jenkins et al., 1970

TABLE V-2 (cont.)

Route	Species/Strain	Sex	Number	Compound	Exposure Level	Duration	Effect	Reference
Inhalation	rat/NS	M	25	mixed xylene (xylene isomers and ethylbenzene)	3500 mg/m^3 (810 ppm)	6 hours/day, 5 days/week x 13 weeks	no effects on weight gain, hematology, blood chemistry or tissue histology were evident	Carpenter et al., 1975
Inhalation	rat/NS	M	25	mixed xylene (xylene isomers and ethylbenzene)	2000 mg/m^3 (460 ppm)	6 hours/day, 5 days/week x 13 weeks	no effects on weight gain, hematology, blood chemistry or tissue histology were evident	Carpenter et al., 1975
Inhalation	rat/NS	M	25	mixed xylene (xylene isomers and ethylbenzene)	770 mg/m^3 (180 ppm)	6 hours/day, 5 days/week x 13 weeks	no effects on weight gain, hematology, blood chemistry or tissue histology were evident	Carpenter et al., 1975
Inhalation	rat/Wistar	M	20	xylene (85% m- and 15% other isomers)	300 ppm (1300 mg/m^3)	6 hours/day, 5 days/week x 5, 9, 14 and 18 weeks	decreased brain superoxide dismutase, after 5 weeks; increased superoxide dismutase by 18 weeks; slight decrease in behavioral activity	Savolainen et al., 1979
Inhalation	guinea pig/NR	NR	≥3	m-xylene	300 ppm (1300 mg/m^3)	4 hours/day, 6 days/week x 11 weeks	slight degeneration of the liver; pulmonary inflammation	Smyth and Smyth, 1928
Inhalation	guinea pig/NR	NR	≥3	m-xylene	300 ppm (1300 mg/m^3)	4 hours/day, 6 days/week x 10 weeks	moderate renal tubular degeneration; 2 cases of chronic catarrhal pulmonary inflammation	Smyth and Smyth, 1928
Inhalation	guinea pig/Princeton	M/F	15	o-xylene	3358 mg/m^3	8 hours/day, 5 days/week x 6 weeks	no effect on body weight gain, histology leukocyte count, hemoglobin or hemotocrit	Jenkins et al., 1970
Inhalation	guinea pig/Princeton	M/F	15	o-xylene	337 mg/m^3	continuous exposure for 90 days	no effect on body weight gain, histology leukocyte count, hemoglobin or hemotocrit	Jenkins et al., 1970
Inhalation	dog/Beagle	M	2	o-xylene	3358 mg/m^3	8 hours/day, 5 days/week x 6 weeks	neuromuscular tremors in one animal; no effect on body weight or hematologic parameters	Jenkins et al., 1970

TABLE V-2 (cont.)

Route	Species/Strain	Sex	Number	Compound	Exposure Level	Duration	Effect	Reference
Inhalation	dog/Beagle	M	2	o-xylene	337 mg/m³	continuous for 90 days	no apparent effects on weight gain or hematologic parameters	Jenkins et al., 1970
Inhalation	dog/Beagle	M	4	mixed xylene (xylene isomers and ethylbenzene)	3500 mg/m³ (810 ppm)	6 hours/day, 5 days/week x 13 weeks	no effect on hematologic parameters or clinical chemistry; no effect on body weight gain, kidney or liver weights	Carpenter et al., 1975
Inhalation	dog/Beagle	M	4	mixed xylene	2000 mg/m³ (460 ppm)	6 hours/day, 5 days/week x 13 weeks	no effect on hematologic parameters or clinical chemistry; no effect on body weight gain, kidney or liver weights	Carpenter et al., 1975
Inhalation	dog/Beagle	M	4	mixed xylene	770 mg/m³ (180 ppm)	6 hours/day, 5 days/week x 13 weeks	no effect on hematologic parameters or clinical chemistry; no effect on body weight gain, kidney or liver weights	Carpenter et al., 1975
Inhalation	squirrel monkey/NA	M	2	o-xylene	3358 mg/m³	8 hours/day, 5 days/week x 6 weeks	no effect on body weight or tissue histology compared with controls	Jenkins et al., 1970
Inhalation	squirrel monkey/NA	M	3	o-xylene	337 mg/m³	continuous for 90 days	no effect on body weight or tissue histology compared with controls	Jenkins et al., 1970
Subcutaneous	rat/Albino	M	9	xylene	0, 172, 430, 860 mg/kg/day	28 days	no change in memory of maze route; dose-related decrease of mean body weight	Desi et al., 1967
Subcutaneous	rat/Albino	M	16	xylene	430 mg/kg/day	6 weeks	decreased learning rate; 20% decreased mean body weight	Desi et al., 1967

NS = Not specified in available abstract; NR = Not reported groups of 10 rats each were exposed to m- or o-xylene at 4300 or 7300 mg/m³ (1005 or 1531 ppm), respectively, for the same exposure period. All xylene isomers at these exposure levels had no apparent effects in mice or rats. It appeared, however, that toxic effects were determined by gross or behavioral signs; microscopic examination of the treated animals was not performed.

In more recent studies, Savolainen and coworkers (Savolainen et al., 1979; Savolainen and Pfaffli, 1980) studied the effects of m-xylene and a mixture of the xylene isomers on male Wistar rats. Brain enzyme and glutathione levels were studied in an effort to determine the mechanism of xylene neurotoxicity. m-Xylene administered at 212, 1710 and 3220 mg/m³ for 6 hours/day, 5 days/week for 2 weeks caused decreased brain glutathione and increased NADPH-diaphorase levels, while treatment at the higher two levels also caused decreased brain superoxide dismutase and an increase in azoreductase activity (see Table V-2) (Savolainen and Pfaffli, 1980). Brain superoxide dismutase levels were decreased after 5 weeks but subsequently increased by 18 weeks of treatment with xylene isomers at 300 ppm (1300 mg/m³), 6 hours daily for 5 days/week (Savolainen et al., 1979). These authors also reported a decrease in preening activity among the treated rats during weeks 9-12 of treatment. Brain levels of protein, RNA, acid proteinase and NADPH-diaphorase did not appear to be affected by xylene treatment. Brain cytosolic glutathione peroxidase activity was decreased ($p<0.05$) compared with that in untreated control rats after 14 weeks of treatment, but returned to near control levels by 18 weeks (Savolainen et al., 1979).

Male rats of the CFY strain (n=30) were exposed to o-xylene by inhalation at 3500 ppm (15,190 mg/m³), 8 hours/day (presumably 7 days/week) for 6 weeks (Tatrai and Ungvary, 1980). (Although the exposure level was reported as 3500 ppm, the authors of this document feel that this may be in error and that 3500 mg/m³ seems more appropriate.) A group of 30 rats inhaling pure air in a similar environment served as controls. Exposed rats consumed more food and water but had decreased body weight gains. Hepatomegaly was evident in exposed rats at necropsy. Hepatocytes, observed histologically, had enlarged nuclei and had larger cells in exposed animals compared with controls. Ultrastructurally, hepatocytes had increased rough and smooth endoplasmic reticulum and decreased glycogen content. Histochemically, centrilobular succinate dehydrogenase and glucose-6-phosphatase was decreased. Hepatic changes were regarded as adaptational.

The effects of repeated daily exposure to mixed xylenes (65.0, 7.6, 7.8% m-, o- and p-xylene and 19.3% ethylbenzene) were determined in rats (n=25/group) and dogs (n=4/group) treated by inhalation at three exposure levels for 6 hours/day, 5 days/week for 13 weeks (Carpenter et al., 1975). Following treatment at 3500, 2000 and 770 mg/m³ (810, 460 and 180 ppm), hematological parameters (blood cell counts, hematocrit and hemoglobin) of rats appeared normal when determined after 3, 7 and 13 weeks of treatment. BUN, SGOT, SGPT and SAP in rats were also not altered by repeated mixed xylene inhalation. Microscopic examination of tissues (lung, liver, kidney, adrenal, brain, pituitary, trachea, thyroid, parathyroid, heart, spleen, stomach, duodenum, pancreas, ileum, jejunum, colon, skeletal muscle, sciatic nerve and bone marrow) of exposed and control rats identified no abnormalities caused by 13 weeks of exposure to the mixed xylene.

In dogs, similarly exposed at 3500, 2000 and 770 mg/m³, 6 hours/day, 5 days/week for up to 13 weeks, blood cell counts and clinical chemistry evaluations showed no difference between pre-exposure and postexposure values. None of the three exposure groups had statistically different body weight gain, liver weight or kidney weights compared with controls. Electrocardiograms taken before and after the 13-week study were normal in all dogs (Carpenter et al., 1975).

Jenkins et al. (1970) tested the effects of subchronic inhalation of o-xylene in rats, guinea pigs, dogs and monkeys exposed continuously for 90 days at 337 mg/m³ (78 ppm) or at 3358 mg/m³ (780 ppm) for 8 hours/day, 5 days/week for 6 weeks (see Table V-2). Terminal body weight of all species exposed repeatedly or continuously were not different when compared with their respective controls. Hematological parameters (leukocyte count, hematocrit and hemoglobin) of rats, guinea pigs and dogs exposed to o-xylene were not different from normal values compiled from pre-exposure data. After necropsy, histopathological examinations of lung, liver, kidney, heart, spleen, brain and spinal cord (nervous tissue examined in dogs and monkeys only) were reported as essentially negative. On the third day of exposure at 3358 mg o-xylene/m³ two rats died while another rat and one monkey died on the seventh day of reported exposure. One rat exposed continuously to o-xylene died on day 56. One of the dogs exposed to o-xylene at 3358 mg/m³ exhibited neuromuscular tremors throughout the study (Jenkins et al., 1970).

Slight degeneration of the liver and pulmonary inflammation were reported in guinea pigs exposed to m-xylene (redistilled from commercial m-xylene) by inhalation. Exposure was initially set at 450 ppm, but a death among the test animals on the first day caused the researchers (Smyth and Smyth, 1928) to decrease the concentration to 300 ppm (1300 mg/m^3). Exposure was 4 hours/day, 6 days/week for a total of 64 exposures. When similar studies were repeated for a total of 58 exposures, slight chronic catarrhal inflammation of the lungs and moderate renal tubular degeneration were noted in guinea pigs exposed to m-xylene at 300 ppm (1300 mg/m^3, 4 hours/day, 6 days/week). Smyth and Smyth (1928) also reported that all 4 rats exposed at 980 ppm for 7 days showed "marked effects," while rats (n=8) exposed at 620 ppm for 7 days showed no ill effects. Further details on these studies, originally conducted by the Benzol Committee, were not reported.

Desi et al. (1967) speculated that the effect of xylene on the CNS may lead to impaired learning and memory ability and, therefore, tested the performance of rats exposed to the solvent in maze running. Two studies were conducted; the first measured the performance of rats already knowing the maze route following xylene exposure (effect on memory), while the second studied the effect of xylene on the ability of rats to learn the maze. In the first experiment, four groups of nine rats each were exposed to 172, 430 and 860 mg xylene/kg/day or a saline solution by subcutaneous injection for 28 days. In the second study, 16 rats were given 430 mg xylene/kg/day by subcutaneous injection for 6 weeks. A vehicle control group of 16 rats was used for comparison. In the first study, no decrement in performance in the maze was evident, but exposed rats did show decreased weight gains in a dose-related pattern. Four of nine animals in the group exposed at 860 mg/kg/day died on the 20th day of injection, at which time this group was discontinued. Demonstrated in the second study, xylene exposure caused significantly ($p<0.02$) slower learning of the maze route. At the end of this study (6 weeks), the mean body weight of the exposed rats was decreased by 20%.

Chronic exposure to o-xylene by inhalation at 4750 mg/m^3 for 8 hours/day, 7 days/week for a 1-year period caused several toxic responses in exposed male CFY rats (Tatrai et al., 1981). Water and food consumption of exposed rats was increased throughout the 1-year study. Hepatic enzyme induction and hepatotoxicity was evident in the treated rats. Hepatic cytochrome P-450 and b_5 concentrations, NADPH-cytochrome c reductase activity, aniline hydroxylase activity and aminopyrin-N-demethylase activity were increased, while bromsulphalein retention time in the liver was decreased. Decreased body weight gain and hepatomegaly were reported. Histologically the livers of exposed rats appeared normal. Upon ultrastructural examination, centrilobular hepatocytes appeared to have moderate proliferation of smooth endoplasmic reticulum and depletion of glycogen, with occasional mitochondrial damage and increased number of autophagic bodies and peroxisomes. Proliferation of the endoplasmic reticulum is consistent with increased enzymes of the mixed function oxidase system. o-Xylene was regarded as an inducer of hepatic enzyme metabolism, and liver enlargement was due to functional hypertrophy during the chronic exposure (Tatrai et al., 1981).

Target Organ Toxicity

Effects attributable to xylene exposure have been predominantly reported in the nervous system, lungs and liver. Effects on the nervous system and lungs are usually reported after acute xylene exposure, while hepatic effects are associated with long-term exposure.

Effects on the Nervous System. Prostration, depression of nervous system, and neuromuscular disorders have been reported as the first signs of intoxication in rats exposed to xylene by inhalation during acute, high level exposures (Carpenter et al., 1975; Furnas and Hine, 1958). At levels above ~10,000 mg/m³ (2300 ppm) for about 3 hours duration, xylene inhalation results in prostration, while exposure at 5800 mg/m³ for up to 4 hours caused altered coordination. No effects were evident after exposure at 2500 mg/m³ for 4 hours (Carpenter et al., 1975).

In two subchronic studies (Savolainen et al., 1979; Savolainen and Pfaffli, 1980) the effect of m-xylene on brain glutathione and biotransformation enzymes was examined (see Table V-2). Brain glutathione levels were decreased, NADPH-diaphorase and azoreductase increased, and superoxide-dismutase was decreased after 5 weeks but elevated by 18 weeks of treatment.

In an attempt to compare fluidization and interaction with lipids and proteins of the axonal membrane with that caused by ethanol and other solvents, xylene was administered to rats for up to 18 weeks with subsequent isolation and biochemical analysis of spinal cord axonal membranes (Savolainen and Seppalainen, 1979). Spinal cord and axonal membranes were isolated by gradient ultracentrifugation. Male Wistar rats were exposed to 300 ppm (1300 mg/m³) xylene for 6 hours/day, 5 days/week for up to 18 weeks. Xylene exposure caused a decrease in axonal membrane lipid content relative to protein, but the cholesterol:lipid phosphorus ratio was unchanged. Lipid solvation may be indicated, but a specific target compound was not suggested.

Oedkvist et al. (1979) reported that xylene causes a positional nystagmus opposite that caused by alcohol exposure. About 30 ppm xylene in the blood (mg/kg) of treated rabbits was necessary to elicit the vestibular disturbance. The response to rotatory acceleration was exaggerated and suggestively caused by blocking of inhibitory nerve pathways, resulting in the stimulation of the central vestibular pathways. Oedkvist et al. (1979) suggested that gamma-aminobutyric acid (GABA), is the transmitter involved with cerebellar inhibition, may be affected by xylene and similar solvents.

The effects of o-, m- and p-xylene and mixed xylene (2.0, 64.5, 10.0 and 23%; o-, m- and p-xylene and ethylbenzene, respectively) on the dopamine and noradrenalin nerve terminal systems were studied in the diencephalen and telencephalon regions of the male rat brain (Andersson et al., 1981). Male Sprague-Dawley rats (n=6) were exposed to the isomers or mixed xylene at 2000 ppm (8680 mg/m^3) for 6 hours/day on 3 consecutive days and killed 16-18 hours after the last exposure. After exposure, dopamine and noradrenalin levels were determined by measuring catecholamine fluorescence. Effects of the xylene on the adenohypophyseal hormones prolactin, corticosterone, follicle stimulating hormone, growth hormone and thyroid stimulating hormone, were assessed by measuring their levels in the serum. Mixed xylene caused a significant ($p<0.05$) increase in the dopamine levels within certain regions of the forebrain, while the specific isomers given separately had no apparent effect (Andersson et al., 1981). Within specific areas of the hypothalamic region of the brain, significant increases in noradrenaline were noted for all xylene isomers and mixed xylene. Following analysis of sera for hormonal levels, significantly ($p<0.05$) decreased levels were noted for thyroid stimulating hormone after mixed xylene treatment, for corticosterone after m- and p-xylene treatment, and for prolactin after treatment with o- and p-xylene. The effects of the xylene isomers and mixed xylene on neurotransmitters may be due to changes in the phospholipid axonal membrane fluidity caused by the solvents, therefore resulting in changes, nerve cell stimulation and transmitter release (Andersson et al., 1981). Changes in neurotransmission within the areas of the brain affected by dopamine and noradrenalin may lead to disturbances in mental and motor functions as well as neuroendocrine control.

Pradhan and Copeland (1983) reported preliminary data which indicate that xylene inhalation by rats causes a depression of self-stimulation behavior. Rats were equipped with wires for electrical intracranial stimulation that could be triggered by depressing a switch within their cage. Following xylene inhalation (exposure not specified), self-stimulation behavior was depressed; this was reversed after exposure was terminated.

Effects on the Lungs. Effects on the lungs and respiratory system are usually reported in acute studies (see Acute Toxicity Section) in which xylene was administered at high concentrations. Difficulty in breathing was reported in rats exposed to xylene by inhalation at 20,000 ppm for 2 hours and in rats exposed for four intervals at 10,000 ppm for 153 minutes total duration (50, 45, 18 and 40 minutes, respectively) (Furnas and Hine, 1958). It is not clear whether these effects were due to pulmonary irritation and congestion or were due to nervous system depression. The authors did report histological evidence of respiratory tract irritation. Similarly, irritation of the respiratory tract was noted in rats treated with 12,000 mg/m³ (2760 ppm) mixed xylene for up to 3.5 hours (Carpenter et al., 1975). This study also reported that 6/15 animals that died after exposure at 43,000 mg/m³ (within 135 minutes) had pulmonary edema, hemorrhage or atelectasis. In addition to pulmonary pathologies, Carpenter et al. (1975) reported that xylene at levels >1300 ppm (5600 mg/m³) caused a 50% reduction in respiratory rate.

In one early subchronic study by Smyth and Smyth (1928), pulmonary inflammation was reported in guinea pigs exposed by inhalation to 300 ppm (1300 mg/m³) m-xylene for 4 hours/day, 6 days/week for 10-11 weeks. Corroborative data for pulmonary pathologies in this or other animal species following subchronic xylene inhalation were not found.

Effects on the Liver. Many studies have identified the ability of the xylene isomers to induce hepatic biotransformation enzymes. These studies are summarized in the Mechanisms of CNS Effects Section in Chapter VII of this report and will not be reviewed here. Also as a result of hepatic metabolism and conjugation (see Metabolism Section in Chapter III), liver glutathione levels were reduced to 26% of pretreatment levels after o-xylene treatment (425 mg/kg by intravenous injection) (Van Doorn et al., 1980).

Effects on the livers of animals are predominantly reported in studies that exposed animals to xylene at low levels for nonacute durations (see Subchronic and Chronic Toxicity Section). Smyth and Smyth (1928) reported slight degeneration in the livers of guinea pigs treated by inhalation with

300 ppm (1300 mg/m³) for 4 hours/day, 6 days/week for 11 weeks. Jenkins et al. (1970) and Carpenter et al. (1975) reported no apparent effects on the livers of rats, guinea pigs, dogs or monkeys following exposure at levels near 3500 mg/m³, 8 or 6 hours/day, 5 days/week for 6 or 13 weeks, respectively.

Exposure to o-xylene at 4750 mg/m³, 8 hours/day, 7 days/weeks for 1 year caused hepatotoxicity in treated rats (Tatrai et al., 1981). Hepatic enzyme induction was demonstrated and treated rats developed hepatomegaly. Electron microscopic examination identified moderate proliferation of smooth endoplasmic reticulum, glycogen depletion and occasional mitochondrial damage, and increased peroxisomes and autophagic bodies in centrilobular hepatocytes.

In a similar study of the ultrastructural changes in livers of rats treated with o-xylene, Bowers et al. (1982) reported effects in rats treated subchronically by oral ingestion. Aging Long-Evans rats (12-19 months old; 800-900 g bw) were fed a diet containing 200 mg o-xylene/kg for up to 6 months. Hepatocytes of these rats developed two types of intercellular vesicles not seen in control animals. Appearing after 1 month of exposure, one vesicle type was thought to be derived from the smooth endoplasmic reticulum and usually was found in the center of the hepatocyte. The second vesicle type usually was located peripherally and appeared to be formed from infoldings of the plasmalemma. The former vesicles may be a response to functional proliferation of metabolic enzymes of the liver and the second type of vesicle may act as a means for increased cell surface area for metabolite elimination. No effects, even at the ultrastructural level, were evident in young rats (3 months old) treated by intraperitoneal injection with 73 mg o-xylene/kg bw on 3 consecutive days.

Other Effects

Carcinogenicity. Three studies have investigated the effect of xylene as a skin irritant in conjunction with a carcinogenic stimulus. None can be used to determine if xylene is carcinogenic.

A skin painting study done on white mice (Berenblum, 1941) compared the carcinogenicity of benzopyrene dissolved in acetone, xylene and turpentine, with and without croton resin. The methods section in this early (1940) publication is not clear. It appears that in the xylene "control" group, undiluted xylene was applied to a 1.5 cm^2 area on 40 mice weekly for 25 weeks, at which time observation was concluded. One tumor in one mouse was seen in 36 survivors at 20 weeks. The tumor was small and regressed entirely. The term "tumor" was used to denote a papilloma at the site of application. Tumors were not examined histologically. The author did not report how frequently the animals were examined for tumors nor did he note the number of tumors or their location.

In another mouse skin painting study (Pound and Withers, 1963), several "irritants" were administered concomitantly with urethane. No controls of nonurethane treated animals were used. The experiment used male "Hall strain" mice. The experimental design was quite complex and was intended to measure the effect of pretreatment with an irritant substance on urethane-induced carcinogenesis. This was done by the administration of 0.25 mℓ of undiluted xylene to the right side of the back on 1-6 days before injection with urethane at the midline of the back. The back was then treated with croton oil once a week for 20 weeks. The authors concluded that pretreatment with xylene increased the yield of tumors. The right side of the back had increased tumor yield compared with the left. There was increasing tumor yield with decreasing interval between the irritant administration and urethane injection. In contrast, acetone had no effect on tumor yield. This experiment provides no information on the carcinogenicity of xylene, because no group of animals was treated with xylene but not urethane.

A third mouse skin painting experiment was performed on "Hall strain" randomly bred white male mice. Pound (1970) studied the effect of pretreatment with xylene on the initiation of skin tumors by UV irradiation. Preliminary treatment consisted of 0.25 mℓ xylene on the left side of the animal followed by UV exposure for 60 seconds. Croton oil (0.25 mℓ croton oil) was administered once a week for 30 weeks thereafter. Acetic acid and croton oil were also used for pretreatment in this experiment. There were 40 animals in each experimental group. Animals were examined for tumors every 5 weeks; the number of tumors and their location were noted. In mice treated with xylene and UV irradiation, there were no tumors in 22 mice surviving 35 weeks. However, in mice treated with xylene but not UV, and promoted with croton oil, there were four tumors in 29 mice alive at 35 weeks. This is not convincing evidence that xylene had the effect of increasing tumor yield because 5/24 mice surviving 35 weeks, who were treated with croton oil alone, had tumors. In mice treated with xylene, UV and croton oil, there were nine tumors in 16 mice surviving 35 weeks. Pretreatment ith acetic acid or croton oil also increased tumor yield. Pound concluded nat cells that had been induced to proliferate were more susceptible to the nduction of tumors by UV light than normal cells. From these skin painting xperiments, it is not possible to conclude whether or not xylene is arcinogenic.

NTP (1983) conducted a carcinogenicity bioassay on xylene administered o rats and mice by gavage, but this study is incomplete. Based on a status eport, the bioassay has been conducted and histopathological examination of experimental animals is in progress. A complete and final report of the study is not available at this time.

Mutagenicity. The limited number of mutagenicity studies on the xylenes indicate that the specific isomers are nonmutagenic (Table V-3). Florin et al. (1980) reported that m- and p-xylene had no activity in initiating reversions of the mutant strains TA98, TA100, TA1535 and TA1537, of Salmonella typhimurium. Strains TA1535 and TA1537 were tested with 0.03, 0.3, 3 and 30 μmol/plate (3, 32, 320 and 3200 μg/plate) of m- and p-xylene in the presence or absence of a microsomal activation system prepared from livers of Aroclor 1254-induced rats. Strains TA98 and TA100 were tested similarly, but were tested additionally using an activation system prepared from 3-methylcholanthrene-induced rat liver. Both m- and p-xylene were toxic to the bacteria at 30 μmol/plate (3200 μg/plate) (Florin et al., 1980).

TABLE V-3

Microbial Mutagenicity Assays on Xylene

Assay/Organism	Strain	Metabolic Activation	Compound	Dose	Type of Application	Response[f]	Reference
Salmonella typhimurium	TA98	+/- S-9[a]	m-xylene	3, 32, 320 and 3200 μg/plate[c]	plate	-	Florin et al., 1980
	TA100	+/- S-9[a]	m-xylene		plate	-	
	TA1535	+/- S-9[b]	m-xylene		plate	-	
	TA1537	+/- S-9[b]	m-xylene		plate	-	
Salmonella typhimurium	TA98	+/- S-9[a]	p-xylene	3, 32, 320 and 3200 μg/plate[c]	plate	-	Florin et al., 1980
	TA100	+/- S-9[a]	p-xylene		plate	-	
	TA1535	+/- S-9[b]	p-xylene		plate	-	
	TA1537	+/- S-9[b]	p-xylene		plate	-	
Salmonella typhimurium	TA98	+/- S-9[d]	o-xylene	20, 50, 100, 200 and 500 μg/plate	plate	-	Bos et al., 1981
	TA100	+/- S-9[d]	o-xylene		plate	-	
	TA1535	+/- S-9[d]	o-xylene		plate	-	
	TA1537	+/- S-9[d]	o-xylene		plate	-	
	TA1538	+/- S-9[d]	o-xylene		plate	-	
Salmonella typhimurium	TA98	+/- S-9[d]	m-xylene	20, 50, 100, 200 and 500 μg/plate	plate	-	Bos et al., 1981
	TA100	+/- S-9[d]	m-xylene		plate	-	
	TA1535	+/- S-9[d]	m-xylene		plate	-	
	TA1537	+/- S-9[d]	m-xylene		plate	-	
	TA1538	+/- S-9[d]	m-xylene		plate	-	
Salmonella typhimurium	TA98	+/- S-9[d]	p-xylene	20, 50, 100, 200 and 500 μg/plate	plate	-	Bos et al., 1981
	TA100	+/- S-9[d]	p-xylene		plate	-	
	TA1535	+/- S-9[d]	p-xylene		plate	-	
	TA1537	+/- S-9[d]	p-xylene		plate	-	
	TA1538	+/- S-9[d]	p-xylene		plate	-	
Escherichia coli	WP2[e]	+/- S-9[b]	technical xylene	starting at 1314 or 1752 μg/well with 11, 2-fold dilutions	microtiter plate and glass tubes	-	McCarroll et al., 1981
	WP2 uvrA	+/- S-9[b]	technical xylene			-	
	CM611	+/- S-9[b]	technical xylene			-	
	WP67	+/- S-9[b]	technical xylene			-	
	WP100	+/- S-9[b]	technical xylene			-	
	W3110	+/- S-9[b]	technical xylene			-	
	p3478	+/- S-9[b]	technical xylene			-	

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[a] With/without S-9 preparations from Aroclor 1254 and 3-methylcholanthrene-induced rat liver

[b] With/without S-9 preparation from Aroclor 1254-induced rat liver

[c] 3200 μg/plate was a toxic dose

[d] With/without S-9 prepared from both untreated and Aroclor 1254 induced rat liver

[e] The minimal concentration for growth inhibition for WP2 was 700 μg/well, all other strains at 1000 μg/well. Well volume was 250 μl.

[f] "-" = Negative

All three isomers of xylene were tested separately in S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 for mutagenic activity (Bos et al., 1981). Compared with the number of spontaneous reversions, none of the xylene isomers when given at 20, 50, 100, 200 and 500 μg/plate were able to cause an increase in the number of histidine-independent revertants. The mutagenic activity of the xylenes was tested in the absence and presence of a rat-liver activation system. Rat-liver preparations were from both untreated rats and Aroclor 1254-pretreated animals.

McCarroll et al. (1981) tested the mutagenic activity of technical xylene (composition not specified) in Escherichia coli mutant strains WP2, WP2 urvA, WP67, WP100, CM611, W3110 (pol A^+) and p3478 (pol A^-). Xylene was tested in a microsuspension plate assay and a glass tube assay for comparison. The dose of xylene applied in either assay was not specified but was stated to be used in 50 μℓ (438 μg) increments per well and serially diluted 2-fold for 11 wells. It was also reported that ~1000 μg/well was the minimal concentration causing growth inhibition of the E. coli strains (strain WP2 was inhibited at 700 μg/well, however). Therefore, assuming at least 1000 μg/well was applied and increments of 50 μℓ xylene were used for the initial, highest concentration well, the highest dose tested would be 1314 or 1752 μg/well (3 or 4, 50 μℓ applications/well, respectively). Well volumes of 250 and 50 μℓ of nutrient medium and activation system were used so that 200 μℓ (1752 μg) xylene is the maximum amount that could be tested per well. Results from both tube and plate assay indicated that xylene was inactive in causing mutant reversion in any of the E. coli strains tested (see Table V-3).

In addition to the previously reviewed microbial mutagenicity assays on xylenes, Donner et al. (1980) reported that m- and o-xylene were not mutagenic in the Drosophila recessive lethal test. Technical xylene, which contained up to 18.3% ethylbenzene, was observed to be weakly mutagenic, causing 1-5% recessive lethals. Pure ethylbenzene, however, did not cause an increase in the recessive lethal frequency above spontaneous levels. Further discussion on the apparent weak mutagenicity of technical xylene was not presented.

Donner et al. (1980) also reported that the number of chromosome aberrations in hematopoietic cells of the bone marrow was not elevated in rats treated with xylene. Rats were exposed to 300 ppm xylene (a mixture of isomers) by inhalation for 6 hours/day, 5 days/week for 9, 14 and 18 weeks. Compared with air-exposed controls, the bone marrow of treated rats had no increase in chromosome breaks. The number of rats exposed and marrow cells analyzed were not reported.

The effect of xylene (composition not specified) on the occurrence frequency of SCEs in human lymphocytes in vitro was examined by Gerner-Smidt and Friedrich (1978). Xylene was tested in whole-blood cultures at 15.2, 152 and 1520 µg/mℓ, with 55-58 metaphase lymphocytes examined at each dose level. The number of SCEs among the first 26 cells in their second mitosis while in culture numbered 221, 194 and 213 in cultures treated at 15.2, 152 and 1520 µg/mℓ, while in untreated culture cells 223 exchanges were reported. Three and two structural chromosome aberrations, respectively, were reported in cultures treated at 15.2 and 1520 µg/mℓ, while none was reported in cells exposed at 152 µg/mℓ and in controls. Growth inhibition (>5% reduction of cell number) was evident in the culture exposed at 1520 mg xylene/mℓ. These authors (Gerner-Smidt and Friedrich, 1978) noted that a metabolic activation system was not used and would be necessary to identify mutagenic metabolites of xylene.

Teratogenicity. The teratogenic and embryotoxic potency of xylenes has been studied in mice following oral administration (Marks et al., 1982) and in mice and rats inhaling the solvent (Shigeta et al., 1983; Hudak and Ungvary, 1978; Ungvary et al., 1980b; Ungvary et al., 1981b). As summarized in Table V-4, mixed xylene (containing xylene isomers and ethylbenzene) was tested by Marks et al. (1982) and Hudak and Ungvary (1978). Specific isomers were tested and ranked according to their embryotoxic potency by Ungvary et al. (1980b).

Mixed xylene (60.2, 13.6, 9.1 and 17.0% of m-, p- and o-xylene and ethylbenzene, respectively) was administered by gavage in cottonseed oil to pregnant CD-1 mice on days 6-15 of gestation (Marks et al., 1982). Xylene solutions (2, 4, 8, 10, 12 and 16% in cottonseed oil) were given at 10 mℓ/kg 3 times daily so that the total daily doses were 0.52, 1.03, 2.06, 2.58, 3.10 and 4.13 g/kg/day. A group of 66 dams received only cottonseed oil and served as a control group. The number of dams treated at each of the dose levels ranged from 15-38 and are specified in Table V-4. Xylene at 4.13 g/kg/day was lethal to all 15 dams, while mortality among mice treated at 3.10 g/kg/day was 12/38. Signs of maternal toxicity were reported in mice treated at 3.1 g/kg/day (decreased maternal weight gain), 2.58 and 2.06 g/kg/day (increased relative liver weights). Fetal weights were also significantly ($p<0.05$) decreased in litters of mice treated at doses of

TABLE V-4

Summary of Teratogenic Effects of Xylenes in Experimental Animals

Route	Species/ Strain	Compound	Dose	Duration	Number of Dams	Maternal Effects	Number of Fetuses	Fetal Effects	Reference
Oral	mice/CD-1	mixed xylene (60.2, 13.6, 9.1 and 17.0% m-, p- and o-xylene and ethylbenzene)	4.13 g/kg/day	days 6-15 of gestation	15	mortality 15/15	0	NA	Marks et al., 1982
Oral	mice/CD-1	mixed xylene (60.2, 13.6, 9.1 and 17.0% m-, p- and o-xylene and ethylbenzene)	3.10 g/kg/day	days 6-15 of gestation	38	mortality 12/38; decreased weight gain; reduced uterine weight; 62% resorptions	79	7 cleft palates; significantly increased ($p<0.01$) percent of malformations; decreased fetal weight ($p<0.05$)	Marks et al., 1982
Oral	mice/CD-1	mixed xylene (60.2, 13.6, 9.1 and 17.0% m-, p- and o-xylene and ethylbenzene)	2.58 g/kg/day	days 6-15 of gestation	28	increased ($p<0.05$) liver weight; 20% resorptions; no deaths	224	11 cleft palates; significantly increased ($p<0.01$) percent of malformations; decreased fetal weight ($p<0.05$)	Marks et al., 1982
Oral	mice/CD-1	mixed xylene (60.2, 13.6, 9.1 and 17.0% m-, p- and o-xylene and ethylbenzene)	2.06 g/kg/day	days 6-15 of gestation	26	increased ($p<0.05$) liver weight; 19% resorptions; no deaths	227	6 cleft palates; 1 exencephaly; significantly increased ($p<0.01$) percent malformations; decreased fetal weight ($p<0.05$)	Marks et al., 1982
Oral	mice/CD-1	mixed xylene (60.2, 13.6, 9.1 and 17.0% m-, p- and o-xylene and ethylbenzene)	1.03 g/kg/day	days 6-15 of gestation	23	no significant signs of maternal toxicity; 16% resorptions	232	1 cleft palate; 2 fused rib arches; no significant increase in percent malformations; fetal weight comparable with controls	Marks et al., 1982
Oral	mice/CD-1	mixed xylene (60.2, 13.6, 9.1 and 17.0% m-, p- and o-xylene and ethylbenzene)	0.52 g/kg/day	days 6-15 of gestation	24	no significant toxic effects; 15% resorptions	208	2 fused sternebrae; no significant increase in malformations; fetal weight comparable with controls	Marks et al., 1982

TABLE V-4 (cont.)

Route	Species/ Strain	Compound	Dose	Duration	Number of Dams	Maternal Effects	Number of Fetuses	Fetal Effects	Reference
Oral	mice/CD-1	none; cottonseed oil only	10 mℓ/kg/day	days 6-15 of gestation	66	11% resorptions; 15.6 gaverage uterine weight; 2.2 g average liver weight; no mortality	658	average 0.3% malformed fetuses; 0.982 g average fetal weight	Marks et al., 1982
Inh.	mice/ICR	xylene	4000, 2000, 1000, 500, 0 ppm, 6 hours/day	days 6-12 of gestation	NS	NR	NS	decreased fetal weight at 2000 and 4000 ppm; decreased neonatal weight gain and hair and teeth growth at 4000 ppm; dose-related delay of sternebrae ossification and 14th rib development	Shigeta et al., 1983
Inh.	rat/CFY	mixed xylene (50, 20, 10 and 20% m-, p-, o-xylene and ethylbenzene)	1000 mg/m^3 (230 ppm), 24 hours/day	days 9-14 of gestation	20	no toxic effects were reported	286	significantly increased fused sternebrae and extra ribs; trend of retarded skeletal development evident	Hudak and Ungvary, 1978
Inh.	rat/CFY	o-xylene	150, 1500, 3000 mg/m^3, 24 hours/day (35, 350, 700 ppm)	days 7-14 of gestation	20, each dose group	significantly increased relative liver weight ($p<0.05$) at all doses	217, 242, 234, respectively, at three dose levels	decreased fetal weight in higher two dose groups ($p<0.001$); 13% fetal loss at highest dose level; skeletal retardation evident at highest dose level	Ungvary et al., 1980b
Inh.	rat/CFY	m-xylene	150, 1500, 3000 mg/m^3, 24 hours/day	days 7-14 of gestation	20, 20, 30, respectively	significantly reduced maternal weight gain and implantations/dam at highest dose level; mortality 4/30 at highest dose level	198, 247, 196, respectively, for dose groups	significantly reduced fetal weight ($p<0.01$) at highest dose level	Ungvary et al., 1980b

TABLE V-4 (cont.)

Route	Species/ Strain	Compound	Dose	Duration	Number of Dams	Maternal Effects	Number of Fetuses	Fetal Effects	Reference
Inh.	rat/CFY	p-xylene	150, 1500, 3000 mg/m³, 24 hours/day	days 7-14 of gestation	20, each dose group	significantly reduced mean litter size and placental weight at highest dose ($p<0.001$)	157, 197, 51, respectively, for dose groups	significantly reduced fetal weight at highest dose level ($p<0.001$); 69% fetal loss (resorption) at highest level; skeletal retardation evident at all dose levels	Ungvary et al., 1980b
Inh.	rat/CFY	p-xylene	3000 mg/m³, 24 hours/day	day 10 or 9-10 of gestation	20, each treatment schedule	significantly decreased levels of peripheral estradiol and progesterone ($p<0.05$) after 48 hours of exposure	NS	decreased fetal weight after 48 hours of exposure	Ungvary et al., 1981b

NA = Not applicable; NR = Not reported; NS = Not specified; Inh. = Inhalation

≥ 2.06 g/kg/day of mixed xylene. The number of implantation sites was not affected by xylene but resorptions were increased in a dose-related fashion, significantly >2.58 g/kg/day ($p<0.05$). Wavy ribs were prevalent in the fetuses of dams treated at 2.58 and 3.10 g/kg/day and incidences of cleft palates were increased at xylene levels of ≥ 2.06 g/kg/day. There was a significant ($p<0.001$) dose-dependent trend for increased percent of malformed fetuses (Marks et al., 1982). Doses of 1.03 and 0.52 g/kg/day had no apparent effects on fetal or maternal toxicity.

In a brief communication, Shigeta et al. (1983) reported data from a fetotoxicity study of xylene (formulation or isomer not specified) in mice (ICR strain) treated by inhalation. Exposure was at 500, 1000, 2000 and 4000 ppm (115, 230, 460 and 920 mg/m^3) for 6 hours/day on days 6-12 of gestation. On day 17 of gestation, two-thirds of the dams in each group (number not specified) were sacrificed and their uteri and fetuses were examined. Uteri were examined for implantation and resorption sites, while fetuses were weighed and examined for external and skeletal malformations. The remaining dams were allowed to deliver and the neonates were observed for abnormal development. The number of implantation sites, resorbed fetuses and dead fetuses were not different between any of the exposure groups and an air-exposed control group. External malformations were not different between any exposure group and control group. Fetal weights were decreased in mice exposed at 2000 and 4000 ppm (460 and 920 mg/m^3) xylene and a dose-dependent trend of delayed ossification and development of a 14th rib was reported (Shigeta et al., 1983). Among the neonates exposed at 4000 ppm (920 µg/m^3), decreased weight gain and delayed body hair and tooth development were attributed to xylene exposure. Additional specific details concerning the methodology and data of this study were not available in this brief report (Shigeta et al., 1983).

The embryotoxicity of mixed xylene (50, 20, 10 and 20%, m-, p- and o-xylene and ethylbenzene, respectively) was tested in CFY rats exposed by inhalation continuously from day 9-14 of gestation (Hudak and Ungvary, 1978). Twenty pregnant rats were exposed at 1000 mg/m^3 (230 ppm), and air-exposed (n=26) and untreated (n=28) controls were used for comparison. Maternal weight gain, mean litter size, mean placental weight and mean fetal weights were not different between exposed and control groups. Fetal loss from resorbed and dead fetuses was 5.30% (16/286) among xylene-exposed dams, while that in air-exposed and untreated controls were 4.13 and 3.37%, respectively. This difference, however, was not statistically significant. Fused sternebrae (8/286) and extra ribs (9/286) were significantly ($p<0.05$) more prevalent and an increasing trend of retarded skeletal development was evident among xylene-exposed rats when compared with controls. Visceral malformations were not increased among the xylene-exposed fetuses (Hudak and Ungvary, 1978).

The embryotoxic potencies of the three xylene isomers were determined and compared in studies conducted by Ungvary et al. (1980b) (Table V-5). Pregnant CFY strain rats were exposed by inhalation to o-, m- or p-xylene vapor (analytical purity) at 150, 1500 or 3000 mg/m^3 (35, 350 or 700 ppm) continuously on days 7-14 of gestation. The number of rats exposed in each isomer concentration group was 20, except for m-xylene at 3000 mg/m^3 (700 ppm), which contained 30 rats. A group of 15, 25 or 20 pregnant rats

TABLE V-5

Comparative Embryotoxic and Maternotoxic Effects of Xylene Isomers Following Inhalation in CFY Rats[a]

Parameter	Inhalation of Compound Continuously on Days 7-14 of Gestation											
	o-Xylene (mg/m^3)				m-Xylene (mg/m^3)				p-Xylene (mg/m^3)			
	Air	150	1500	3000	Air	150	1500	3000	Air	150	1500	3000
No. of females:												
treated	15	20	20	20	25	20	20	30	20	20	20	20
nonpregnant	2	3	2	1	2	5	1	8	2	8	4	7
total litter resorption	0	0	0	2	0	0	0	0	0	0	0	7
died	0	0	0	0	0	0	0	4	0	0	0	0
Maternal weight gain (% starting weight)	57.5	60.0	57.2	54.3	65.8	67.3	64.7	48.3[b]	52.9	58.2	54.5	41.3
Liver weight/bw (%)	3.88	4.00[b]	4.24[b]	4.18[b]	4.27	4.35	4.45	4.67	4.12	3.98	4.08	4.56
No. of litters	13	17	18	17	23	15	19	18	18	12	16	6
No. of fetuses:												
live	168	217	242	234	284	198	247	196	226	157	197	51
resorbed	14	17	14	35	27	17	14	11	9	8	13	115
dead	3	0	1	0	0	0	3	1	0	0	1	0
Mean implantations/dam	14.2	13.8	14.3	14.2	13.5	14.3	13.9	11.4[b]	13.1	14.1	13.4	12.8
Mean litter size	12.9	12.8	13.4	13.8	12.4	13.2	13.0	10.9	12.6	13.1	12.3	8.5[c]
Fetal loss (% of implantation sites)	9.2	7.3	5.8	13.0	6.7	7.9	6.4	5.3	4.0	9.0	8.0	69.0[d]
Mean fetal weight (g)	4.07	4.02	3.72[c]	3.75[c]	3.97	3.85	3.94	3.60[d]	4.05	4.02	3.97	3.56[c]
Mean placental weight (g)	0.63	0.61[b]	0.65[b]	0.68[c]	0.65	0.65	0.65	0.69	0.73	0.64[d]	0.66[c]	0.61[c]

[a] Source: Ungvary et al., 1980b

[b] $p<0.05$

[c] $p<0.001$

[d] $p<0.01$

exposed to clean air served as controls for the o-, m- or p-xylene-exposed groups, respectively. Exposures were conducted in inhalation chambers (0.4 m^3 volume) at a flow rate of 5 m^3/hour at regulated temperature (24-25°C) and humidity (50-55%). Animals were sacrificed on day 21 of gestation and parameters indicative of maternal and fetal toxicity were measured. Signs of maternal toxicity included decreased food consumption after exposure at 1500 mg/m^3 (o-xylene only) and 3000 mg/m^3 (all three isomers), mortality (m-xylene only), decreased weight gain and increased relative liver weight. Decreased fetal weights were evident in groups exposed to 1500 mg/m^3 (o-xylene only) and at 3000 mg/m^3 (all isomers). Postimplantational fetal loss due to resorption was significantly ($p<0.01$) increased in rats exposed to p-xylene at 3000 mg/m^3. Pre-implantational loss of embryos, indicated by an increase in nonpregnant dams or a decrease in the number of implants per pregnant rat, was increased in dams exposed to 3000 mg m-xylene/m^3 and p-xylene at all concentrations.

Macroscopic examinations were conducted on fetuses to identify external, visceral and skeletal malformations (Table V-6) (Ungvary et al., 1980b). The most prominent skeletal abnormalities were "signs of skeletal retardation" (assumed to be delayed ossification) and extra ribs. No increase in gross external, soft tissues or skeletal malformations was reported. Histological examinations of "representative organs," enzymatic determinations of the liver, kidney and thymus, and electronmicroscopic studies of the liver were conducted on 5, 3 and 3 representative fetuses, respectively, from each isomer/concentration group. No xylene-related histological changes were observed in any group. Renal alkaline phosphatase (proximal convoluted

TABLE V-6

Abnormalities of Fetuses of Rats Treated with o-, m- or p-Xylene[a]

Parameter	Continuous Inhalation by Dams on Days 7-14 of Gestation											
	o-Xylene (mg/m³)				m-Xylene (mg/m³)				p-Xylene (mg/m³)			
	Air	150	1500	3000	Air	150	1500	3000	Air	150	1500	3000
No. of live fetuses	168	217	242	234	284	198	247	196	226	157	197	51
Gross external malformations	0	1[b]	0	0	0	0	0	0	0	0	0	1[b]
No. of fetuses examined internally	84	107	118	113	139	95	118	94	110	80	96	25
Soft tissue malformations:												
pulmonary polycysticus	0	1	0	0	5	2	3	0	1	0	0	0
pyelectasia	11	7	10	17	5	2	2	8	3	3	11	3
ectasia ves. urin.	0	1	5	9	3	5	0	5	0	0	0	1
No. of fetuses examined skeletally[c]	84	110	123	121	142	102	123	97	116	77	100	26
Skeletal retardation signs	13	27	30	48[d]	6	4	6	15	16	24[d]	38[d]	15[d]
Skeletal anomalies:												
fused sternum	0	3	4	0	1	0	0	0	1	0	1	2
extra ribs	0	2	3	3	2	1	3	8[d]	6	1	9	10[e]
Skeletal malformations	0	1[b]	0	0	0	0	0	0	0	0	0	[illegible]

[a] Source: Ungvary et al., 1980b

[b] Agnathia

[c] Alizarine-red S staining for skeletal examination

[d] $p<0.05$ (statistical procedure used in the analysis was the Mann-Whitney U-test)

[e] $p<0.01$ (statistical procedure used in the analysis was the Mann-Whitney U-test)

tubules), succinic dehydrogenase, acid phosphatase and glucose 6-phosphatase (nephrons) appeared less in fetuses from dams exposed to o- and p-xylene at 3000 mg/m³ (700 ppm) compared with controls. All three isomers at 3000 mg/m³ (700 ppm) decreased fetal hepatic and thymic succinic dehydrogenase and glucose-6-phosphatase. The decreased levels of renal, hepatic and thymic enzymes were regarded as indicative of retardation of maturation of the enzyme synthesis system.

In an attempt to determine whether p-xylene-induced embryotoxicity could be attributed to its effects on ovarian and uterine circulation and hormone production, Ungvary et al. (1981b) treated pregnant CFY rats on day 10 or days 9 and 10 of gestation. p-Xylene was administered by inhalation at 3000 mg/m³ (700 ppm) to 20 animals for 24 hours/day. Two hours after exposure was terminated, rats were anesthetized and the uteri and ovaries were cannulated through the utero-ovarian veins. Blood flow and levels of 17β-estradiol and progesterone were determined. The uteri were then removed and weighed and the embryos counted and weighed. Ovarian blood flow appeared lower after 48 hours of exposure, but the decrease was not statistically significant. Uterine blood flow was not changed. Fetal weights decreased with exposure duration, significantly ($p<0.01$) so after 48 hours, but placentas appeared normal (gross and histologically). Ovarian progesterone and estradiol secretion rate was not significantly affected by p-xylene exposure for either duration. Levels of progesterone and estradiol detected in the uterine vein were significantly ($p<0.05$) decreased in rats exposed for 48 hours. Ungvary et al. (1981b) concluded that the delayed fetal development was not due to altered uterine circulation, and ovarian hormone production was not affected, but peripheral hormone levels were decreased.

The p-xylene may, therefore, cause more rapid hormone metabolism by the hepatic mixed function oxidase system, leading to decreased peripheral hormone titers and the embryotoxic effects.

A study conducted by Overman (1981) on the embryotoxic effects of xylene and other laboratory reagents indicates that minimal effects are noted in the embryos, while effects on maternal skin were more extensive. Xylene (isomer or mixture not specified) was applied to the shaved backs of hamsters for exposure periods of 2 hours between days 7 and 11 of gestation. It is not clear whether xylene was applied to the skin on one or all days throughout the period because the report was limited in scope. The applied dose and number of animals treated are not explicated. Effects reported generally for all reagents tested included decreased fetal size and weight, increased prenatal death and unspecified cases of fetal hemorrhage and gastroschisis.

Summary

Based on the available studies in laboratory animals, xylene is regarded as having a low toxicity. Oral LD_{50} values in rats ranged from 3.6-6.75 g/kg for the specific isomers and 4.3-6.1 g/kg for a xylene mixture. The LC_{50} values of m-, o- and p-xylene were 5267, 4595 and 3907 ppm (22,860, 19,940 and 16,960 mg/m^3) following 6 hours of inhalation in mice (Bonnet et al., 1979). In rats, the LC_{50} for 4 hours of inhalation was 6350-6700 ppm (27,600-29,100 mg/m^3) (Hine and Zuidema, 1970; Carpenter et al., 1975).

The toxicity of o-, m- and p-xylene following acute oral exposure has been examined in rats (Ungvary et al., 1981a; Pyykko, 1980) and rabbits

(Bray et al., 1949). Ungvary et al. (1981a) treated male and female rats with 2.2 g/kg/day for 4 days and reported 20% mortality and diapedetic hemorrhage in several organs among females. Decreased body weight gains, increased liver weights and centrilobular hypertrophy were noted in both sexes following exposure to all three isomers. Pyykko (1980) treated only male rats with 1.1 g/kg/day of each isomer for 3 days. All rats appeared to behave normally, but mean liver weights were increased following exposure to all isomers. Data on the toxic effects of acute orally ingested xylene in rabbits were reported by Bray et al. (1949). No toxic effects were evident after 1.3 g of o-, m- or p-xylene was administered per rabbit, and 1.75 g/rabbit was considered a toxic dose because of excessive amounts of urinary "reducing material."

Exposure to a pure mixture of xylene isomers by inhalation at 4000 ppm (17,360 mg/m³) for 2 hours had no toxic effect on rats (Furnas and Hine, 1958). Two hours of exposure at 20,000 ppm (87,000 μg/m³) caused difficulty in breathing, ataxia, muscular twitching and spasms. Carpenter et al. (1975) reported that 2500 mg mixed xylenes/m³ (575 ppm) for 4 hours caused no signs of distress or toxicity in rats. At 5800 mg/m³ (1333 ppm) rats exhibited poor coordination, and concentrations of $\geq$12,000 mg/m³ ($\geq$2759 ppm) caused prostration or death within the 4-hour exposure period. Effects on the lungs were suggested as the cause of death in rats following xylene exposure (26,000 and 43,000 mg/m³ for up to 4 hours).

In a short-term inhalation study in rats, Savolainen and Pfaffli (1980) exposed animals to m-xylene at 212, 1710 and 3220 mg/m³ for 6 hours/day, 5 days/week for 2 weeks, and measured levels of brain enzyme and glutathione.

Exposure at all levels caused decreased glutathione and increaed NADPH-diaphorase levels, and at the higher two concentrations of m-xylene caused decreased brain superoxide dismutase and azoreductase activities. In another study by Savolainen et al. (1979), exposure at 300 ppm (1300 mg/m³), 6 hours/day, 5 days/week for 18 weeks produced no consistent effects on brain enzyme and glutathione levels in rats.

Male rats exposed to o-xylene at 3500 ppm (15,190 mg/m³), 8 hours/day for 6 weeks (presumably 7 days/week) developed hepatomegaly (Tatrai and Ungvary, 1980). Histologically, hepatocytes had enlarged nuclei and increased cell size. Ultrastructural changes in endoplasmic reticulum and decreased glycogen content, as well as hepatic histochemical changes, were also reported.

Carpenter et al. (1975) conducted an inhalation study in which rats and dogs were exposed to mixed xylene at 770, 2000 and 3500 mg/m³ for 13 weeks. Hematological, blood chemistry and extensive histological examinations were performed, but no abnormalities were identified in the treated rats and dogs. In another subchronic inhalation study, Jenkins et al. (1970) reported no toxic effects in rats, guinea pigs, dogs and monkeys exposed at 337 mg/m³ (78 ppm) continuously for 90 days or at 3358 mg/m³ (780 ppm) for 8 hours/day, 5 days/week for 6 weeks. Hematological parameters and histopathological examinations of major organs were essentially negative.

Pulmonary inflammation and slight degeneration of the liver were reported in guinea pigs exposed to redistilled m-xylene at 300 ppm (1300 mg/m³) for 4 hours/day, 6 days/week for 64 exposures (Smyth and Smyth, 1928). When similar studies were reported with o-xylene at 300 ppm on the same exposure schedule for 58 exposures, catarrhal inflammation of the lungs and renal tubule degeneration were diagnosed in treated guinea pigs (Smyth and Smyth, 1928).

Desi et al. (1967) studied the effects of xylene by subcutaneous injection on memory and learning in rats. A group of rats knowing the maze were treated with 172, 430 or 860 mg xylene/kg/day for 28 days, but no apparent effects on maze performance occurred. Other rats, not familiar with the maze, showed significantly impaired ($p<0.02$) learning ability after treatment at 430 mg xylene/kg/day for 6 weeks. This 6-week treatment also caused marked (20%) decrement of rat body weight.

The toxic effects of o-xylene administered subchronically in the diet were studied in male rats (Bowers et al., 1982). Rats were exposed for 1-6 months at 20 mg o-xylene/kg of diet. Gross and histological examinations revealed no effects attributable to o-xylene. Electronmicroscopic studies revealed hepatocyte changes and the appearance of two types of vesicles, consistent with increased hepatic metabolism of the administered o-xylene.

In a chronic inhalation study, o-xylene was administered at 4750 mg/m^3 for 8 hours/day, 7 days/week for 1 year to male CFY rats (Tatrai et al., 1981). Hepatotoxic effects were reported with increased hepatic enzyme activities (cytochrome P-450, cytochrome b-5, NADPH-cytochrome c reductase, aniline hydroxylase and aminopyrin-N-demethylase), hepatomegaly and decreased body weight gain. Ultrastructural examination showed proliferation of smooth endoplasmic reticulum of centrilobular hepatocytes and glycogen depletion, while histologically, livers appeared normal. The primary effect of o-xylene was described as hepatic enzyme induction, which resulted in noted ultrastructural changes and liver enlargement by functional hypertrophy.

The target organs that are most notably affected are the nervous system, lungs and liver. The nervous system and respiratory system are affected during acute, high-level exposures, while hepatic effects appear following more long-term exposure. Xylene inhalation at levels ≥2300 ppm (10,000 mg/m³) for ~3 hours caused prostration in rats, while incoordination was exhibited by rats after 4 hours of exposure at 5800 mg/m³ (1300 ppm) (Carpenter et al., 1975). Xylene has been shown (Savolainen and Seppalainen, 1979) to decrease the axonal membrane lipid content in central and peripheral nervous tissue, which possibly causes the anesthetic and narcotic effect noted following acute exposures. Oedkvist et al. (1979) also suggested that xylene may affect the normal function of the brain neurotransmitter, GABA. Xylene isomers and mixed xylene caused increased dopamine and noradrenalin levels in certain regions of the brain in rats treated for 3 days (6 hours/day) at 2000 ppm (8680 mg/m³) (Andersson et al., 1981).

Effects of xylene on the lungs and respiratory tract usually include irritation, congestion, edema and hemorrhage. Such effects were reported in rats treated at 12,000 mg/m³ (2760 ppm) for up to 3.5 hours, and those dying after 135 minutes of exposure at 43,000 mg/m³ (Carpenter et al., 1975). Smyth and Smyth (1928) reported pulmonary inflammation in guinea pigs exposed to 300 ppm (1300 mg/m³) m-xylene for 64 exposures (4 hours/day, 6 days/week).

Xylene has extensive effects on liver. Van Doren et al. (1980) reported reduced liver glutathione levels as a result of metabolism and conjugation. Xylene has been shown to be an inducer of hepatic enzymes. This inductive effect was thought to be the cause of hepatomegaly and ultrastructural changes noted in hepatocytes of rats inhaling o-xylene at 4750 mg/m^3 (1092 ppm) for 8 hours/day, 7 days/week for 1 year (Tatrai et al., 1981). Bowers et al. (1982) reported the formation of two types of vesicles within hepatocytes of rats treated orally with 200 mg o-xylene/kg of diet for up to 6 months. One vesicle type was felt to originate from smooth endoplasmic reticulum in response to increased hepatic enzyme production, while the second vesicle type was associated with the plasmalemma and possibly acted in metabolite exocytosis.

One available study on the dermal carcinogenicity of xylene reported negative results in mice (Berenblum, 1941), while others did not provide conclusive information (Pound and Withers, 1963; Pound, 1970). A recent carcinogenicity bioassay on xylene administered by gavage to rats and mice is currently in progress but a final report is not available at this time (NTP, 1983).

The available mutagenicity studies on the xylene isomers or technical xylene indicates that these compounds are not mutagenic. Tests with _Salmonella_ _typhimurium_ strains TA98, TA100, TA1535 and TA1537 conducted with and without activation systems at 3, 32, 320 and 3200 µg/plate (3200 µg/plate was a toxic dose) were negative for m- and p-xylene (Florin et al., 1980). Bos et al. (1980) also reported negative results when o-, m- and p-xylene were tested in strains TA98, TA100, TA1535, TA1537 and

TA1538 at 20, 50, 100 and 500 g/plate. Xylene was inactive in causing mutant reversion in *E. coli* strains WP2, WP2 urvA, WP67, WP100, CM611, W3110 (pol A^+) and p3478 (pol A^-) at concentrations high enough to cause growth inhibition (1000 µg/well) (McCarroll et al., 1981). Donner et al. (1980) reported that technical xylene was weakly mutagenic in *Drosophila* recessive lethal tests, while pure o- and m-xylene were not mutagenic. In rats treated with xylene for up to 18 weeks (300 ppm, 6 hours/day, 5 days/week), chromosome aberrations were not increased in bone marrow cells (Donner et al., 1980). *In vitro*, xylene caused no increase in the number of SCEs in human lymphocytes, but metabolic activation was not used (Gerner-Smidt and Friedrich, 1978).

Based on the available teratogenicity and embryotoxicity studies, xylene appears to be embryotoxic and possibly causes increased malformations. Mixed xylene administered orally to mice increased the percent of fetal malformations (cleft palates) and decreased fetal weight at doses of $\geq$2.06 g/kg/day (administered on days 6-15 of gestation) (Marks et al., 1982). Following inhalation exposure to rats (24 hours/day on days 7-14 of gestation), fetal weights were reduced ($p<0.01$) at 1500, 3000 and 3000 mg/m^3, respectively, for o-, m- and p-xylene, and skeletal retardation was evident in fetuses (Ungvary et al., 1980b). Ungvary et al. (1981b) reported that fetal weights were decreased following exposure at 3000 mg p-xylene/m^3 for 48 hours on days 9-10 of gestation. With the exception of the oral study by Marks et al. (1982) where cleft palates were increased, the teratogenicity studies revealed generally retarded skeletal development and body weight gains in fetuses.

VI. HEALTH EFFECTS IN HUMANS

The effects of xylenes on humans have been determined in laboratory exposure experiments with volunteers and through case reports and epidemiology studies of occupationally exposed workers.

Laboratory Studies in Human Volunteers

A fairly extensive study of the effects of p-xylene (99% purity, <1 ppm benzene) on male and female volunteers was conducted by Hake et al. (1981) for the National Institute for Occupational Safety and Health. Male subjects were exposed at 20, 100 and 150 ppm for 1 (n=2-3), 3 (n=2) and 7.5 (n=4) hours/day for 5 consecutive days at each exposure level. Males were sedentary except for those exposed for 7.5 hours/day, who exercised for 11 minutes on day 4 of each exposure week. Females were exposed while sedentary to 100 ppm p-xylene for 1 (n=2), 3 (n=2) or 7.5 (n=3) hours/day on 5 consecutive days. Parameters tested to identify toxic effects included hematological profile, urinalysis, EEGs, VERs, cardiopulmonary functions, cognitive performance, time-estimation performance, coordination, visual recognition performance, arithmetic performance and subjective complaints. Measurements taken during the exposure intervals were compared with pre- and postexposure values in men and women. No significant abnormalities attributable to p-xylene exposure were reported in EEGs, VERs, pulmonary ventilation volume, heart rate, metabolic rate, blood pressure or any of the cognitive or performance tests in men or women. Except for an increased level of methylhippuric acid, urinalysis and hematology were normal. The authors (Hake et al., 1981) did note the possible accumulation of p-xylene in the blood of males during periods of exercise with exposure at 150 ppm.

Two male subjects showed a decreased performance in the Flanagan coordination test following 7.5 hours of exposure to 150 ppm p-xylene, while the other two subjects exposed at this level performed normally. This decrement in performance was not considered to be dose-related. Based on the small number of subjects, this effect cannot be attributed positively to p-xylene exposure, nor discounted as a toxic effect. Subjective complaints included eye, nose and throat irritation from one subject who wore contact lenses and from another male, both exposed for 7.5 hours at 100 and 150 ppm p-xylene. Nose and throat irritation and headache were also reported in the females exposed at 100 ppm (7.5 hours) for 5 days. Hake et al. (1981) concluded that p-xylene exposure at 100 ppm was near a no-effect-level in sedentary males, but sensitive individuals may experience eye and upper respiratory tract irritation. More serious effects may be seen in females or during exercise at levels near 100 ppm.

Irritation of the eyes, nose and throat was the major complaint from 10 subjects (both sexes) exposed to xylene (purity or mixture not specified) at 200 ppm for 3-5 minutes (Nelson et al., 1943). Based on these very brief exposures, a majority of subjects felt that exposure at 100 ppm would be tolerable for an 8-hour workday.

Groups of 6 or 7 human volunteers were used in a study by Carpenter et al. (1975) to determine the sensory threshold levels of mixed xylenes (65.0, 7.8, 7.6 and 19.3% m-, p-, o-xylene and ethylbenzene, respectively) when inhaled at 0.46, 1.0, 2.0 or 3.0 mg/ℓ (110, 230, 460 or 690 ppm). Volunteers inhaled the solvent at a different concentration for one 15-minute period on 4 consecutive days. No significant effects (eye or throat irritation, lacrimation, dizziness or tasting of the compound) were reported by the group of six subjects exposed at 0.46 mg/ℓ. After the 15-minute exposure at 1.0 mg/ℓ, 1/7 subjects reported eye irritation and lacrimation, tasting the solvent and dizziness. Eye irritation was the predominant complaint, reported by 4/6 subjects, following exposure at 2.0 mg/ℓ. In addition to eye and throat irritation and lacrimation, 4 of 6 subjects exposed to 3.0 mg/ℓ mixed xylene (690 ppm) reported dizziness. All subjects reported sensing the odor of the solvent at levels $\geq$0.06 mg/ℓ, and the average olfactory threshold level was determined to be near 0.0045 mg/ℓ (1.0 ppm) (Carpenter et al., 1975). Hake et al. (1981) also reported that p-xylene at 1.0 ppm could be detected by smell.

The effect of mixed xylene (54.4, 12.8, 12.1 and 20.7% m-, p-, o-xylene and ethylbenzene) inhalation on central nervous function was determined by the performance of male subjects in 5 tests (addition simple and choice reaction time, short-term memory and critical flicker fusion frequency) (Gamberale et al., 1978). Two series of experiments were conducted. In the first, three groups of 5 subjects each were exposed at rest to 0, 435 or 1300 mg mixed xylene/m^3 for 70 minutes and exposures were alternated every other day so that each group was exposed at all three levels. In the second experiment, eight of the same subjects were exposed to 1300 mg/m^3 mixed xylene, and exercised (bicycle ergometer at 100 W work) for the first 30 minutes of the study. The xylene vapor disguised with menthol was administered by a breathing valve, and vapor concentration was monitored by hydrocarbon analyzer and gas chromatography. Performance testing was conducted during the last 35 minutes of the exposure period. In the first experiments (exposure while at rest), no significant changes in performance in any test were found in subjects exposed at 435 or 1300 mg/m³. Following exposure at 1300 mg/m³ and exercise, the total uptake of xylene was notably increased (1210 mg uptake versus 180 and 541 mg, respectively, at rest) and performance in all five tests was impaired. Significant decreases ($p<0.05$) in performance were found for addition reaction time (time needed for the subject to add 4 displayed numbers and report the result) and short-term memory (recall and report the sequence of 4-10 numbers displayed to the subjects). Gamberale et al. (1978) noted that exercise increased the uptake of xylene and presumably elevated blood levels of the solvent to a level that caused a depressant effect on neural function. It was also noted that the performance of more complex and cognitive tests showed more decrement and that neural pathways for these mental tasks may be more sensitive.

Savolainen et al. (1980a) conducted psychophysiological performance tests on 8 male volunteers exposed to m-xylene at constant and varied concentrations (90-188 ppm TWA, 6 hours/day, at rest and while exercising 100 W on bicycle ergometer). Reaction time, manual dexterity, body balance and EEG were recorded on 5 consecutive days and 1 additional day after the weekend (2-day nonexposure period). Body balance was impaired following exposure to constant xylene at 90 ppm (3 hours of exposure in the morning, 1 hour lunch break and 3 hours of exposure in the afternoon) on the first day of exposure and on the first day following the weekend. Tolerance to the xylene was evident. Reaction time and manual coordination were impaired at various random times during the exposure week. When xylene concentrations were fluctuated from 64-200 ppm (93 ppm TWA), body balance was impaired on the third day of exposure and on the fifth day when exposure levels were doubled. Only slight and random changes in the reaction times of subjects were noted throughout this exposure. Changes in the spontaneous EEG (increasing number of occipital slow transient waves) were reported in four subjects exposed to xylene when the concentrations were varied from 64-200 ppm.

The effects of m-xylene (laboratory grade) inhalation for 4 hours at ~6.5 and 11.1 μmol/ℓ (160 and 280 ppm) were tested in two groups of 5 male volunteers (Savolainen et al., 1980b). Each group was tested at both exposure levels during different weeks. Blood levels of xylene and body sway, visual-manual reaction time, and tapping speed were determined before and after exposure and twice during the exposure period. Mean m-xylene blood levels reached 22.1 and 40.7 μmol/ℓ (2.35 and 4.32 mg/ℓ) following exposure at the low and high concentrations, respectively. m-Xylene exposure at either concentration had no significant effect on the average body sway, but the maximum amplitudes of sway showed a tendency to increase following m-xylene exposure. Choice reaction time (visual stimuli converted to manual choice) was not affected by m-xylene at ~6.5 μmol/ℓ, but 11.1 μmol/ℓ showed some impairment, although not statistically so. Simple reaction time and tapping speed were not impaired following exposure at either concentration of m-xylene (Savolainen et al., 1980b). In further tests of the same group of volunteers exposed on the same schedule, Savolainen et al. (1980b) additionally reported that the critical flicker fusion threshold (the frequency at which strobosonic light pulses appear as continuous illumination) was not impaired by xylene at the lower concentration and improved in subjects at ~11.1 μmol/ℓ (1175 mg/m^3). Extraocular muscle balance and ocular nystagmus was not affected by exposure to m-xylene at either concentration tested.

The effects of short-term m-xylene inhalation on body balance and reaction time were further examined by Riihimaki and Savolainen (1980). Male volunteers (n=22) were exposed to m-xylene for 6 hours/day on 5 consecutive days followed by a 2-day nonexposure weekend and 1-3 more days of exposure. Xylene concentrations were either constant (at 100 except elevated to 200 ppm on afternoon of day 5) or varied from baseline levels (70 or 130 ppm) to peak levels (200 or 400 ppm) so that TWA concentrations were 100 or 200 ppm. Subjects were sedentary during exposure to constant levels of m-xylene and exercised during peak exposures in experiments in which m-xylene concentrations were varied. Body balance (measured by body sway amplitude) and reaction time (choice selection time) were impaired following exposures at 400 ppm in sedentary subjects. Development of tolerance to m-xylene was indicated by a decrease in the effects of exposure during later days of the week of exposure, but discernible effects were noted after the weekend. After exercise, body balance, reaction time and dexterity (Santa Ana test) were affected by m-xylene exposure when peak levels of 400 ppm were reached (Riihimaki and Savolainen, 1980). The effects of m-xylene were relatively short-lived with only occassional impairment of body balance, lasting until 0.5 hours after exposure was terminated.

Savolainen et al. (1982a,b) exposed 9 male volunteers, 20-25 years of age, to m-xylene at 8.2 μmol/ℓ (200 ppm) and measured the effect of exposure on balance and body sway. Body sway (anteroposterior and lateral movement) was determined using a strain gauge transducer attached to the platform on which subjects stood. Subjects were tested for body balance with eyes closed and opened. Because vision is important in maintaining body posture, even control subjects showed a greater amount of body sway with eyes closed. The eyes closed:eyes open ratio of body sway was used as a measure of nonvisual balance control. Exposure was 4 hours/day, 1 day/ week and tests were conducted over 6 consecutive weeks. Average changes in the ratio of body sway with eyes closed:eyes open were reported for subjects during exposure days and nonexposure days (control ratio). This study showed a significant ($p<0.05$) decrease in the ratio of body sway with eyes closed versus eyes open when compared with nonexposure values (Savolainen et al., 1982a,b). This finding was opposite that determined in an earlier study (Savolainen and Linnavuo, 1979) in which six subjects showed increased body sway after m-xylene exposure. In the earlier report, Savolainen and Linnavuo (1979) reported that m-xylene at 100 ppm (TWA with 200 ppm peaks) had no significant effect on body sway and equilibrium following 3 hours of exposure during the morning. After subsequent afternoon exposures at 200 ppm (TWA, with 400 ppm peaks), balance and body sway were significantly impaired in the anteroposterior axis ($p=0.016$) and lateral axis ($p=0.016$) when the eyes were closed. Lateral body sway was also significantly ($p=0.03$) increased when the eyes were open. Quantitatively, controls had 4.50, 2.90 and 1.48 mm of anteroposterior, lateral (eyes closed), and lateral (eyes open) movement, respectively, while subjects exposed to 100 ppm m-xylene in the morning and 200 ppm during the afternoon had 6.42, 3.72 and 1.65 mm of movement, respectively. Anteroposterior movement with eyes open was slightly increased but not significantly so after m-xylene exposure. Because of the opposite effect of m-xylene on body sway determined in these two studies (Savolainen and Linnavuo, 1979; Savolainen et al., 1982a,b), it was suggested in the later publication that m-xylene may have a biphasic pattern of acute anesthetic effect. Stimulation and depression of the CNS by m-xylene may be critically dependent on brain and blood levels of the compound, therefore resulting in increased or decreased body sway at different periods (Savolainen et al., 1982a). Savolainen and Riihimaki (1981b) reported that only fluctuating xylene concentrations (up to 400 ppm) could elicit an increase in the ratio of body sway with eyes closed:eyes open. This effect was attributed to the rapid rise in blood xylene levels during peak concentrations, affecting the central nervous equilibrium maintenance function but not visual pathways.

Clinical Case Reports

Three cases of acute poisoning by xylene were reported by Morley et al. (1970). Three men, aged 24, 52 and 54, were painting an enclosed tank within a ship, apparently became unconscious while working and were not rescued until ~18.5 hours later. Xylene represented >90% of the paint solvent (solvent was 34% of paint weight) and toluene-based cleaning solvent was available but not used. Based on conditions of the tank and the amount of paint applied, xylene concentration was approximated at 10,000 ppm. One man died as a result of exposure and had severe pulmonary congestion, hemorrhage and edema, hepatic congestion, cellular swelling and vacuolization, and petechial hemorrhage of the brain. The second man was unconscious for a period of 5 hours and was confused and amnesic for 2-3 days thereafter. This patient showed evidence of hepatic injury (raised serum transaminase levels) for at least 2 days and renal function was impaired for at least 15 days. The third man was unconscious but recovered soon after rescue; however, confusion, ataxia and amnesia persisted for up to 48 hours. No renal impairment was apparent but liver cell damage was suggested by elevated serum transaminase levels for 48 hours postexposure (Morley et al., 1970).

Klaucke et al. (1982) reported 15 cases of illness among hospital laboratory employees, which apparently resulted after 1 ℓ of xylene was discarded down a drain. The ventilation unit of the laboratory area had drawn the xylene vapor into the work area. Illness had developed within 15-75 minutes of the xylene disposal. The workers' symptoms included headache (12/15), dizziness (7/15), eye irritation (8/15), upper respiratory irritation (7/15), nausea (10/15) and vomiting (6/15); headaches persisted for up to 48 hours. Based on a simulation of this accident and a review of human exposures, xylene levels were estimated to be 700 ppm.

Five and four additional cases of xylene poisoning were reported by Hipolito (1980) and Angerer and Lehnert (1979), respectively. All cases were female cytotechnologists or histology technicians who worked with xylene in the preparation and cleaning of histological slides. For the cases reported by Hipolito (1980), levels of xylene vapor to which workers were exposed were not determined or estimated but exposure durations ranged from 1.5-18 years. Symptoms common to the 5 women were headache, cyanosis, thoracic pains, electrocardiogram abnormalities, decreased pulmonary function, leukopenia, dyspnea, nausea and vertigo. These symptoms are consistent with those of xylene toxicity and in three cases an expert diagnosis of xylene-related illness was made (Hipolito, 1980).

The four women reported by Angerer and Lehnert (1979) were exposed to m-and p-xylene at 56-68 ppm, o-xylene at 10-13 ppm and ethylbenzene at 34-41 ppm in the laboratory. These workers had no complaints attributable to the solvent exposures.

Two cases of xylene-induced epileptiform seizures have been reported on two separate occassions (Goldie, 1960; Arthur and Curnock, 1982). In one case, an 18-year-old male had an epileptic seizure following exposure to xylene-containing paint (Goldie, 1960); a boy experienced a seizure following use of xylene-based model airplane glue (Arthur and Curnock, 1982). In the first report (Goldie, 1960) eight painters, one of whom suffered an epileptiform seizure, were exposed to paint containing 80% xylene and 20% methylglycolacetate solvent for several months and complained of headache, throat irritation, abdominal pain and dizziness. In the latter case, generalized seizures usually occurred ~24 hours after exposure to xylene, while no seizures occurred if a methyl ethyl ketone/tetrahydrofuran-based glue was used.

As discussed by Browning (1965), alleged cases of leukemia and less severe blood abnormalities previously associated with xylene exposure have, in most cases, been linked to benzene, a contaminant of the xylene. The actual hematotoxic effects of xylene are uncertain, but the pure compound is regarded as having a negligible, if any, effect.

Epidemiological Effects

Mikulski et al. (1972) studied a group of 51 ship painters exposed to xylene (84-93%), toluene (6-16%) and benzene (not detectable-2%), and used

21 unexposed workers for comparison. This study emphasized changes in urinary excretion in workers exposed to <100 ppm (Group I) or >200 ppm (Group II) xylene. Creatinine excretion appeared to be elevated in workers exposed to high solvent levels. Consistent with the metabolism of xylenes, toluene and benzene, urinary glucuronic acid, hippuric acid and phenol were increased in a dose-related manner. Uric acid excretion was reduced in a dose-related manner and was attributed to a reduction in glycine for conjugation. The authors (Mikulski et al., 1972) reported no other xylene-related effects but suggested that inhibition of uric acid production may be important in diseases that also affect the excretion of this metabolite.

In a series of publications, Askergren and coworkers (Askergren et al., 1981a,b; Askergren, 1981, 1982) examined renal functions in workers exposed to various organic solvents. The study group consisted of 134 workers of whom only 40 were exposed to xylene and toluene in the manufacturing of paint. A control group of 48 unexposed workers was used. The mean concentration of urinary albumin among workers exposed to xylene and toluene (13.7 mg/ℓ) was elevated ($p<0.05$) compared with controls (3.7 mg/ℓ), but the median values were comparable (3.2 and 3.1 mg/ℓ, respectively). These data indicate that subjects excreting high levels of albumin were more numerous in the exposed group, but not all subjects had elevated levels. Levels of φ-2-microglobulin and creatinine in the urine and osmolality of the urine were comparable between control and exposed groups (Askergren et al., 1981a). In studies testing the ability of the kidney to concentrate the urine, Askergren et al. (1981b) reported that the group (n=118) exposed to various solvents (styrene, toluene or xylene and toluene) showed no decrement in renal function. Exposure levels of the study group were moderate, exceeding Swedish limits (styrene 170 mg/m^3, toluene 300 mg/m^3 or xylene 350 mg/m^3) by 2-3 times for only short periods, and were generally below the limits (Askergren et al., 1981b). In another study Askergren (1981) reported the excretion of erythrocytes and leukocytes in the urine of men exposed to styrene, toluene, or xylene and toluene. Reported generally for the exposed group, the average excretion of red blood cells and white blood cells in the urine was significantly elevated ($p=0.011$ and $p=0.014$, respectively) when compared with controls. The increase in urinary blood cells was attributed to glomerular damage by the different solvents. Askergren (1982) suggested that increased permeability of the glomerular basement membrane could cause increased albumin and blood cell excretion, while proximal distal tubular function (measured by concentration ability) did not appear to be affected.

Renal damage as a result of solvent exposure was also studied by Franchini et al. (1983) in workers exposed to several types of cyclic and aliphatic hydrocarbons. A group of 118 painters (predominantly males) was exposed to toluene, xylene and other benzene homologues for an average of 9.1 years. Exposure levels of xylene were not determined or estimated. Two control groups consisting of males and females (50 females and 30 males or 16 females and 65 males) were used for comparison. Excretion of albumin in the urine as a marker of increased glomerular permeability was not elevated in the exposed group. Urinary φ-glucuronidase levels in exposed painters were significantly ($p<0.01$) higher than in either control group. Lysozymurea in the painters was elevated but not significantly so when compared with the reference groups.

The activities of liver enzymes in the serum of 102 car painters exposed primarily to xylene and toluene were determined and compared with a group of 102 age-matched controls (Kurppa and Husman, 1982). Overall solvent levels were reportedly about half the TLV recommended by the ACGIH (1980) (100 ppm xylene; 100 ppm toluene). The group of 102 male car painters, from 27 repair shops, was exposed to toluene (mean concentration of 306 ppm), xylene (5.8 ppm) and other solvent components (butyl acetate, 6.8 ppm; white spirit, 4.9 ppm and others) for periods of 1-40 years. Effects of the solvent exposure on serum enzyme activities, neurophysiological function and ophthalmological parameters were also studied (Kurppa and Husman, 1982). The reference group consisted of locomotive engineers and assistants. Twelve of 59 of the car painters examined neurophysiologically had abnormally slow motor and sensory conduction velocities and neuromuscular latencies. Lens opacities were identified in 48/92 car painters having ophthalmological examinations. The results of serum enzyme activity determinations (aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase and ornithine carbamoyl transferase) were comparable between the exposed and referent groups. Because of the complex nature of solvent exposure in this group of workers, it is difficult to determine an etiological agent. In fact, toluene may be of primary importance because of its concentration (Kurppa and Husman, 1982).

A group of 17 paint industry workers exposed to xylene (111 mg/m³) and 10 other solvents was used to study the possible genotoxic activity of these solvents (Haglung et al., 1980). Peripheral lymphocytes were prepared from the painters and a group of matched controls for chromosome studies. The frequency of SCEs and chromosomal aberrations were compared between the two groups. The frequency of SCEs was nearly identical between the two groups (0.193 and 0.192 exchanges per chromosome for painters and controls, respectively). The number of chromosome aberrations (gaps, translocations, deletions, inversions, breaks, aneuploidy) among workers most extensively exposed to the solvents was also not different from their respective referent. No chromosomal abnormalities could be related to solvent exposure (Haglung et al., 1980).

The previously reviewed epidemiological studies can, at best, provide only suggestive evidence of the effects of xylene on workers. It is difficult to draw a conclusion from these studies because of the complex nature of occupational exposure to xylene (usually mixed exposures of many solvents) and the poorly quantified exposure levels.

High Risk Subpopulations

It is obvious that humans exposed to xylene in occupational situations are at elevated risk of toxic effects. Based on the available case reports of xylene toxicity in humans, painters and laboratory workers appear most frequently affected. Painters using xylene-based paints and thinners may be affected during acute or extended exposures (Morley et al., 1970; Askergren et al., 1981a,b). Painting in confined areas seems most dangerous for acute xylene poisoning.

Laboratory workers, where xylene is used as a solvent and dehydration fluid, also have experienced xylene intoxication (Klaucke et al., 1982; Hipolito, 1980). Once again, the use of xylene in improperly ventilated areas was the cause for toxic levels of xylene in the ambient workroom air.

Workers involved in the distillation and purification of xylene or industries using xylene as a raw ingredient (gasoline blending) are also at risk of exposure (Cier, 1970). During normal operations, xylene levels may be low with ample precautions, but accidental spills or careless use can lead to higher exposure levels. Accidental spills may be the major cause of high-level xylene exposure.

Pregnant women, and hence embryos, fetuses and young children, exposed to xylene should be considered a high risk subpopulation (Mirkova et al., 1982). Embryonic and neonatal stages are often more susceptible to toxic chemicals because of the delayed development of hepatic detoxication mechanisms. Kucera (1968) has suggested that exposure to certain fat solvents such as xylene may be associated with birth of children with sacral agenesis. Based on a teratogenicity study in mice (see Teratogenicity in Chapter V), mixed xylene administered orally (2.06 g/kg/day) caused increased resorption, decreased implantations and reduced fetal weights (Marks et al., 1982). Inhalation of xylene also caused decreased fetal weights of mice treated at 2000 and 4000 ppm (6 hours/day on days 6-12 of gestation) (Shigeta et al., 1983). In rats, Hudak and Ungvary (1978) suggested that xylene inhalation (1000 mg/m^3) caused development of extra ribs and fused sternebrae as well as delayed skeletal development. Ungvary et al. (1980) reported that inhalation of o-, m- or p-xylene at 3000 mg/m^3 by pregnant rats caused decreased fetal weights and increased pre- and postimplantational fetal loss. Ungvary et al. (1981b) further suggested that p-xylene exposure may cause rapid hormone catabolism, causing decreased peripheral progesterone and estradiol titers which, in turn, result in decreased fetal development.

In an attempt to correlate congenital defects of the CNS with maternal occupational exposure, Holmberg and Nurminen (1980) obtained records of the Finnish Register of Congenital Malformations and questionnaire information concerning the parents. A total of 120 cases and referents (birth subsequent to defective birth) were studied. This study (Holmberg and Nurminen, 1980) found that most often mothers of defective children had been exposed to a variety of organic solvents (n=13) during the first trimester. In only one case was exposure to xylene listed by the mother. Smoking habits between mothers were comparable. This study does not indicate an assocation of human teratogenicity with xylene but suggests the possibility that pregnant women may be susceptible to chemicals at occupational exposure levels.

Summary

Xylene toxicity in humans has been studied following experimental, accidental and occupational exposure. Several laboratory studies in which volunteers were acutely exposed to xylene by inhalation suggested 100 ppm as a NOAEL (Nelson et al., 1943; Carpenter et al., 1975; Riihimaki and Savolainen, 1980). Hake et al. (1981) reported that sedentary females exposed to 100 ppm xylene for 7.5 hours/day for 5 consecutive days complained of nose and throat irritation and headache. Exercise increased xylene toxicity so that females exposed during exercise (or work) at levels <100 ppm may show toxic effects. The average olfactory threshold was near 1 ppm (0.0045 mg/ℓ) (Carpenter et al., 1975).

Effects of xylene on performance in various cognitive, reaction time and body balance tests have been reported following exposure at 1300 mg/m³ for 70 minutes in conjunction with exercise (Gamberale et al., 1978). Riihimaki and Savolainen (1980) exposed male volunteers to m-xylene at constant and varied levels so that TWA concentrations were 100 and 200 ppm for 5 consecutive days and 1-3 more days following a 2-day nonexposure weekend. Effects on body balance, reaction time and manual dexterity were reported only after exposure at 200 ppm TWA with peak levels at 400 ppm. Savolainen et al. (1982a,b) reported, in opposition to an earlier report (Savolainen and Linnavuo, 1980), that that body sway ratio with eyes closed: eyes open (a measure of non-ocular body balance) was decreased following m-xylene exposure.

Case reports of xylene intoxication have been reported for painters (Morley et al., 1970) and laboratory workers (Hipolito, 1980; Klaucke et al., 1982). Symptoms included headache, dizziness, vertigo, nausea and vomiting; in severe cases (Morley et al., 1970), anmesia, ataxia, confusion, unconsciousness, pulmonary congestion, hepatic congestion, and renal failure, and in one case, death. In two cases, the induction of epileptiform seizures was attributed to xylene exposure (Goldie, 1960; Arthur and Gurnock, 1982). In histology assistants chronically exposed to xylene, headache, cyanosis, thoracic pain, EKG abnormalities, leukopenia, dyspnea, nausea and vertigo were reported (Hipolito, 1980).

Because of the complex nature of occupational xylene exposure (exposure to many chemicals, unspecified xylene exposure levels), epidemiology reports are of little value in quantifying chronic xylene toxicity. Emphasis has been focused on effects of long-term xylene exposure on renal function. In addition to the increased excretion of urinary metabolites of xylene (see Metabolism and Excretion in Chapter III), occupational exposure (painters and paint manufacture workers) has been associated with increased albumin, creatinine, erythrocytes and leukocytes in the urine (Mikulski et al., 1972; Askergren et al., 1981a,b; Askergren, 1981). These factors were attributed to glomerular damage caused by solvent exposure, while proximal and distal tubules appeared to function normally. Franchini et al. (1983) reported opposite effects in a group of painters exposed to toluene and xylene. This study identified normal urinary albumin excretion and suggested normal glomerular function while φ-glucuronidase levels were increased, suggesting tubular damage.

In a study of automobile painters (n=102) exposed to toluene (30.6 ppm), xylene (5.8 ppm) and other solvents, abnormally slow sensory and motor conduction velocity and increased neuromuscular latencies were found in 12 individuals, and lens opacities of the eye were identified in 48 painters. Serum levels of four enzymes (aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase and ornithine carbamoyl transferase) were not different between exposed and referent groups (Kurppa and Husman, 1982).

As can be ascertained from the occupations in which case reports and epidemiological surveys have been conducted, painters and laboratory workers exposed to xylene-based solvents are at high risk. Workers employed in the distillation and purification of xylene or in industries where xylene is used as a solvent or raw ingredient (gasoline blending) would also be at high risk of exposure. Pregnant women, their embryos and fetuses, and neonates should be regarded as groups at increased risk of developing toxic effects from xylene. Animal studies suggest maternal and fetal toxicity, while teratogenicity is not substantiated (Marks et al., 1982), although Kucera (1968) has suggested an associated between xylene exposure and the birth of children with sacral agenesis. No study in humans which would substantiate this concern of increased susceptibility to xylene intoxication in developmental stages and pregnant women was available.

VII. MECHANISMS OF TOXICITY

Toxic effects following xylene exposure have been reported in the CNS, lungs, kidneys and liver. Although a definite mechanism of toxic action is not known, researchers have speculated, based on available data, on the mode of action for each target organ. A summary of the proposed mechanisms of toxicity for each organ (system) is presented here. Data from both animal studies and human exposures have been used to suggest mechanisms of toxicity under the assumption that different species are affected similarly by xylene.

Mechanisms of CNS Effects

Effects on the nervous system, primarily the CNS, stem from the lipophilic nature of xylene. Many researchers have noted the affinity of xylene (a lipid solvent) for nervous tissue because of the high lipid content of myelin and the axonal membrane (Gerarde, 1959; Desi et al., 1967; Savolainen and Pfaffli, 1980). The toxic effects of xylene on the nervous system (anesthesia, narcosis) are noted after acute, high level exposures when high blood and brain levels develop. Gerarde (1959) postulated that xylene and other alkylbenzenes acted on the nervous system merely by being present in these tissues at a concentration that inhibited normal function (physical toxicity). Desi et al. (1967) also noted that the accumulation of xylene in the nervous tissue may interfere with CNS functions, but speculated that xylene may be more effective on brain-stem functions. Studies conducted by Savolainen and coworkers (Savolainen and Linnarvuo, 1979; Savolainen, 1980; Riihimaki and Savolainen, 1980; Savolainen and Riihimaki, 1981b; Savolainen et al., 1982a) have reported that the first observable effects of xylene exposure are on the central vestibular system. More complex nervous pathways, such as those controlling body balance, tended to be affected first

(Savolainen, 1980). Effects on body balance were noted in human volunteers exposed to 200 ppm (TWA, 6 hours/day) during 10-minute peak exposures at 400 ppm (Savolainen and Riihimaki, 1981a).

Savolainen and Pfaffli (1980) suggested that the toxic effects following xylene exposure in rats may be due to intermediate metabolites such as arene oxides or methylbenzaldehyde. Oxidation of xylene to these intermediate metabolites by microsomal enzyme systems can occur within the brain cells (Savolainen and Pfaffli, 1980). In an English abstract of a Bulgarian study, Popov and Ivanova-Chemishanska (1980) also noted that the toxicity of xylene is caused by metabolites, such as the two previously noted, and superoxide anion. These metabolites caused pathologies in tissues where they were formed (brain, lung, kidney and liver).

Xylene may also directly affect nerve function by altering the lipid components of the axonal membrane. This fluidization, or solvation, of the axonal membrane could alter sodium permeability and decrease action potentials, resulting in signs of nervous system intoxication. The effect of xylene exposure on the biochemistry and physiology of rat spinal cord axonal membranes was studied by Savolainen and Seppalainen (1979). Protein and lipid content and composition were determined for spinal cord axon membranes from 10 rats exposed to 300 ppm xylene (6 hours/day, 5 days/week) for 18 weeks and compared with 45 control animals. Axonal membranes isolated from animals exposed to xylene had a decreased lipid to protein content, although the cholesterol to lipid phosphorus ratio did not appear changed. Adaptation to compounds that are capable of fluidizing axonal membranes, such as xylene, may occur by saturation or unsaturation of fatty acids in the axonal membrane. Adaptation to xylene has been shown to occur in humans (Savolainen and Riihimaki, 1981b).

In a study investigating the mechanism by which various solvents, including xylene, cause positional nystagmus through disturbances of the central vestibular system, Oedkvist et al. (1979) suggested that inhibition of vestibular pathways may be blocked. Two drugs, picrotoxin and bicuculline, that affect the CNS and cause a positional xylene nystagmus (PXN) are inhibitors of GABA. This neurotransmitter mediates cerebellar inhibition of vestibular pathways and may be blocked by xylene (Oedkvist et al., 1979).

Studies by Andersson et al. (1981) indicate that acute, high-level xylene exposure can cause changes in brain dopamine and noradrenaline levels, which lead to altered brain function. Rats were treated with mixed xylene (64.5, 10, 2 and 23% m-, p-, o-xylene and ethylbenzene) or the purified (≥98.5%) components separately at 2000 ppm, 6 hours/day for 3 days. Levels of the two catecholamines were determined in sections of the brain at 16-18 hours postexposure (see Effects on the Nervous System, Chapter V). Mixed xylene exposure increased dopamine levels in the forebrain, while none of the separated components produced any effect. In the hypothalamic region all compounds elicited effects on noradrenalin and dopamine content. The rate of catecholamine metabolism (turnover) was also increased by all of the xylene isomers. These authors (Andersson et al., 1981) reported more extensive and widespread effects following m-xylene exposure. Two possible modes of action were suggested by these authors. A metabolite of xylene or xylene could act directly on the adrenergic receptors in the brain, causing increased catecholamines by accumulation and postsynaptic stimulation. The second possibility involves alteration of axonal membrane fluidity [similar to that suggested by Savolainen and Riihimaki (1981b)], which causes permeability changes and alters neurotransmitter release.

Mechanisms of Pulmonary Effects

Pulmonary damage following exposure to xylenes, primarily by inhalation, had led to inquiry into the mode of action in this organ. Gerarde (1959) discussed the effect alkylbenzenes, such as xylene, can have on endothelial cells, especially in the lung following exposure by all routes. Permeability changes in capillaries can lead to edema and petechial or gross hemorrhage. In acute oral toxicity studies (see Acute Toxicity, Chapter V), pulmonary injury and gross hemorrhage was the cause of death in most cases studied (mortality was 7/10, 3/10, 6/10 following ingestion of o-, m- and p-xylene).

Most studies have attempted to identify pulmonary metabolites of xylene that cause enzyme induction and possibily interact with cellular components causing the pulmonary damage. Patel et al. (1978) reported that within 1 day of p-xylene treatment either by gastric intubation (1 g/kg), inhalation (1000 ppm, 4 hours/day) or intraperitoneally (1 g/kg), pulmonary p-xylene hydroxylase was depressed to 30-35% of control levels in treated female Sprague-Dawley rats. Cytochrome P-450 content of pulmonary microsomes was decreased (Toftgard and Nilsen, 1982). NADPH-cytochrome C reductase activity was also significantly ($p<0.01$) reduced following inhalation of p-xylene. Patel et al. (1978) postulated that a hepatic metabolite was responsible for the pulmonary effects and suggested p-tolualdehyde as the active metabolite. In vitro, p-tolualdehyde inhibited pulmonary microsomal enzymes similar to that seen in vivo, while p-xylene required alcohol dehydrogenase to be effective. p-Tolualdehyde was, therefore, concluded to cause the enzymatic changes in the lung. In contrast, Pyykko (1980) reported no changes in pulmonary NADPH cytochrome C reductase, aminopyrine demethylase, aryl hydrocarbon hydroxylase or aniline hydroxylase following oral exposure to any of the xylene isomers (1.1 g/kg/day for 3 days) in male Sprague-Dawley rats.

The metabolism and effects of p-xylene in the lungs were studied in situ in rabbits (Smith et al., 1982). The primary metabolite was p-methylbenzyl alcohol with lesser amounts of 2,5-dimethylphenol. Contrary to the findings of Patel et al. (1978), this study showed no evidence of hepatic metabolites that would cause the destruction of pulmonary cytochrome P-450. Smith et al. (1982) reported that metabolism of p-xylene by the lungs is not complete because of the deficiency of alcohol dehydrogenase in this tissue; the metabolites produced bind to cellular proteins within the lungs. Only cytochrome P-450-dependent monooxygenation occurs and pulmonary-derived p-tolualdehyde may be responsible for protein binding and pulmonary damage.

Mechanisms of Renal Effects

Toxic renal effects may be produced by xylene in a similar manner to effects in the lungs. Increased capillary (glomerulus) or tubular permeability, caused by irritant or fluidization effects, would result in physiological and possibly histological effects. In rats treated by inhalation, xylene has caused increased cytochrome P-450, 7-ethoxycoumarin-O'-deethylase, UDP-glucuronosyltransferase, NADPH cytochrome c-reductase, and n-hexane hydroxylase (Elovaara, 1982; Toftgard and Nilsen, 1982). Relative kidney weight was also elevated significantly ($p<0.05$) by o-xylene at 2000 ppm, 6 hours/day for 3 days (Toftgard and Nilsen, 1982).

Effects of occupational xylene exposure on the kidney and renal function were investigated by Askergren and coworkers (Askergren et al., 1981a,b; Askergren, 1981; Askergren, 1982). A group of 40 paint industry workers exposed mainly to xylene and toluene were studied (see Epidemiological Effects, Chapter VI). A referent group of 48 nonexposed workers was used. Urinalyses were used to assess renal function and led researchers to conclude that glomerular damage was evident while renal tubules appeared to function normally. Workers had higher urinary albumin levels and more blood cells in the urine (evidence of increased glomerular permeability), while urinary osmolality (tubular urine concentrating ability) was normal. The suggested glomerular damage is consistent with endothelial damage suggested by Gerarde (1959) as a result of alkylbenzene exposure.

Franchini et al. (1983) reported contradictory results from studies of renal damage among 118 painters exposed to low levels of toluene and xylene (see Epidemiological Effects, Chapter VI). Renal tubular damage and not glomerular was suggested. This group of workers had significantly increased urinary β-glucuronidase and lysozymuria indicative of slight tubular lesions (Franchini et al., 1983).

Mechanisms of Hepatic Effects

Ultrastructural hepatic effects have been reported in rats following subchronic oral exposure (200 mg/kg diet for up to 6 months) (Bowers et al.,

1982) and chronic inhalation exposure (4750 mg/m³, 8 hours/day, 7 days/ week for 1 year) (Tatrai et al., 1981) to o-xylene. Bowers et al. (1982) reported the presence of two types of intracellular vesicles in rats treated with o-xylene. One type appeared to be an extension of the endoplasmic reticulum and may have resulted from metabolic adaptation to the o-xylene. The second vesicle type was associated with the hepatocyte plasmalemma and, speculatively, represented exocytotic vesicles of o-xylene metabolites (Bowers et al., 1982). These effects, therefore, were regarded as results of enzyme induction and increased hepatic metabolism. Bowers et al. (1982) also reported hepatic nodules on the free margin of the liver in young rats treated with o-xylene by intraperitoneal injection (73 mg/kg) on 3 consecutive days. These lesions contained lipid droplets and were surrounded by fibroblasts and macrophages. Hepatocytes, however, appeared normal. These nodules were considered extrahepatic responses to the compound because of the mode of injection.

Similar to data reported by Bowers et al. (1982), Tatrai et al. (1981) reported hepatomegaly and ultrastructurally evident proliferation of the smooth endoplasmic reticulum. Proliferation of the endoplasmic reticulum is consistent with the increased levels of hepatic enzymes and increased metabolism of the o-xylene. As demonstrated by both these ultrastructural studies and other studies on hepatic enzyme induction (see the Enzyme Induction in the Liver Section), hepatic effects are due to functional adaptation toward xylene detoxication.

Enzyme Induction in the Liver. The studies indicating hepatic enzyme induction following exposure to xylene are numerous (Table VII-1). Following oral administration the three xylene isomers are inducers of dealkylation enzymes and hydroxylases and also cause an increase in the concentration of hepatic cytochrome P-450, cytochrome b_5, and NADPH cytochrome c-reductase (Pyykko, 1980; Ungvary et al., 1981a). The different isomers of xylene have been shown to have slightly differing potencies of induction. For example, only m-xylene significantly ($p<0.001$) increased hepatic cytochrome P-450 content in rats, while o- and p-xylene had little effect following 3 days of ingestion at 1.1 g/kg/day (Pyykko, 1980).

Inhalatory exposure to technical xylene for 2 weeks (6 hours/day, 5 days/week) at 300 ppm caused an increase in 7-ethoxycoumarin o-deethylase activity but did not significantly increase levels of cytochrome P-450, NADPH cytochrome c-reductase, DT-diaphorase, or 2,5-diphenyloxazole hydroxylase (Savolainen et al., 1978). Patel et al. (1979) reported significant ($p<0.05$) increases in the activity of several serum enzymes [SGPT, lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), SGOT, isocitrate dehydrogenase (ICD), glutathione reductase (GSS-Rase), 5'-nucleotidase, pseudocholinesterase (AcChase)] believed to be of hepatic origin. Enzyme levels were altered within 24 hours of the 4-hour exposures (1000, 1500, 2000 ppm). Interpretation of the altered enzyme levels with respect to p-xylene toxicity was as follows: increased SGPT and SGOT indicated hepatocellular damage; G-6-PDH and ICD were increased because of increased metabolism of glucose to produce NADPH; GSS-Rase activity was increased to maintain cellular glutathione levels; LDH may have been liberated from damaged hepatic tissue; AcChase activity may have resulted from

TABLE VII-1

Effects of Xylene Exposure on Hepatic Enzymes and Cytochromes in Animals

Route	Species/ Strain	Number/ Sex	Compound	Exposure Level	Duration	Enzyme	Effect^a	Reference
Oral	rat/ Sprague-Dawley	12/M	o-xylene	1.1 g/kg/day in corn oil	3 days	Cytochrome P-450 Cytochrome b-5 NADPH cytochrome c-reductase Aryl hydrocarbon hydroxylase Aminopyrine N-demethylase Aniline hydroxylase	content unchanged content increased content unchanged activity unchanged activity increased activity unchanged	Pyykko, 1980
Oral	rat/ Sprague-Dawley	12/M	m-xylene	1.1 g/kg/day in corn oil	3 days	Cytochrome P-450 Cytochrome b-5 NADPH cytochrome c-reductase Aryl hydrocarbon hydroxylase Aminopyrine N-demethylase Aniline hydroxylase	content increased content increased content increased activity unchanged activity increased activity increased	Pyykko, 1980
Oral	rat/ Sprague-Dawley	12/M	p-xylene	1.1 g/kg/day in corn oil	3 days	Cytochrome P-450 Cytochrome b-5 NADPH cytochrome c-reductase Aryl hydrocarbon hydroxylase Aminopyrine N-demethylase Aniline hydroxylase	content unchanged content increased content increased activity unchanged activity increased activity unchanged	Pyykko, 1980
Oral	rat/CFY	8/MF	o-xylene	2.2 g/kg/day	4 days	Cytochrome P-450 Cytochrome b-5 NADPH cytochrome c-reductase Aminopyrine N-demethylase Aniline hydroxylase	content increased content unchanged activity increased activity increased activity increased	Ungvary et al., 1981a
Oral	rat/CFY	8/MF	m-xylene	2.2 g/kg/day	4 days	Cytochrome P-450 Cytochrome b-5 NADPH cytochrome c-reductase Aminopyrine N-demethylase Aniline hydroxylase	content increased content unchanged activity increased activity increased activity increased	Ungvary et al., 1981a
Oral	rat/CFY	8/MF	p-xylene	2.2 g/kg/day	4 days	Cytochrome P-450 Cytochrome b-5 NADPH cytochrome c-reductase Aminopyrine N-demethylase Aniline hydroxylase	content unchanged content increased activity increased activity increased activity unchanged	Ungvary et al., 1981a

TABLE VII-1 (cont.)

Route	Species/ Strain	Number/ Sex	Compound	Exposure Level	Duration	Enzyme	Effect[a]	Reference
Inhalation	rat/Wistar	5/M	technical xylene (80, 12% m-, p-xylene)	300 ppm (1300 mg/m³)	2 weeks, 6 hour/day 5 day/week	Cytochrome P-450	content unchanged	Savolainen et al., 1978
						NADPH cytochrome c-reductase	activity unchanged	
						7-ethoxycoumarin o-deethylase	activity increased	
						2,5-diphenyloxazole hydroxylase	activity unchanged	
						DT-diaphorase	activity unchanged	
Inhalation	rat/ Sprague-Dawley	8/F	p-xylene	1000, 1500, 2000 ppm (4340, 6510, 8680 mg/m³)	single 4 hour	SGPT[b]	activity increased	Patel et al., 1979
						SGOT[b]	activity increased	
						Lactate-dehydrogenase[b]	activity increased[c]	
						Glucose-6-phosphate dehydrogenase[b]	activity increased[c]	
						Glutathione reductase[b]	activity increased[c]	
						Isocitric dehydrogenase[b]	activity increased[c]	
						5'-Nucleotidase[b]	activity increased[c]	
						Pseudocholinesterase[d]	activity increased	
Inhalation	rat/CFY	6/M	o-xylene	4500 mg/m³ (1035 ppm)	6 weeks, 8 hour/day	Cytochrome P-450	content increased	Ungvary et al., 1980
						Cytochrome b-5	content increased	
						Aniline hydroxylase	activity increased	
						Aminopyrine N-demethylase	activity increased	
Inhalation	rat/ Sprague-Dawley	4/M	mixed xylene (64.5, 10, 2, and 23% m-, p-, o-xylene and ethylbenzene)	2000 ppm	3 days, 6 hour/day	Cytochrome P-450	content increased	Toftgaard and Nilsen, 1981, 1982
						n-Hexane hydroxylase	activity increased	
						7-Ethoxyresurufin deethylase	activity increased	
						4,5-Benzo-α-pyrene hydroxylation	activity increased	
Inhalation	rat/ Sprague-Dawley	4/M	o-, m-, or p-xylene (pure isomer)	2000 ppm (8680 mg/m³)	3 days, 6 hour/day	Cytochrome P-450	content increased by all isomers	Toftgaard and Nilsen, 1981, 1982
						n-Hexane hydroxylase	activity increased[e]	
						7-Ethoxyresurufin deethylase	activity increased by o-, m-xylene	
						4,5-Benzo-α-pyrene hydroxylation	activity increased by all isomers	
Inhalation	rat/ Sprague-Dawley	4/M	mixed xylene (64.5, 10, 2, and 23% m-, p-, o-xylene and ethylbenzene)	600 ppm (2600 mg/m³)	4 weeks, 6 hour/day 5 day/week	Cytochrome P-450	content not changed	Toftgaard and Nilsen, 1981, 1982
						9,10-Benzo-α-pyrene hydroxylase	activity increased	
						4,5-Benzo-α-pyrene hydroxylase	activity increased	

TABLE VII-1 (cont.)

Route	Species/ Strain	Number/ Sex	Compound	Exposure Level	Duration	Enzyme	Effect[a]	Reference
Inhalation	rat/ Sprague-Dawley	4/M	xylene	630 ppm (2760 mg/m^3)	4 weeks, 6 hour/day 5 day/week	Cytochrome P-450	content increased 20% (NS)	Toftgaard et al., 1981; Toftgaard and Nilsen, 1982
						2-, 4-Biphenyl hydroxylase	activity increased	
						9, 10- or 4,5-Benzo-α-pyrene diol hydroxylase	activity increased	
						4 androstene-3,17-dione	activity increased	
						6 , 7α or 16-hydroxylase	activity increased	
						5 α-androstane-3 , 17 β-diol 2α-hydroxylase	activity increased	
						5 α-androstane-3 , 17 β-diol 7β or 18-hydroxylase	activity increased	
Inhalation	Rat/Wistar	10/M	m-xylene	50, 400, 750 ppm (217, 1736, 3255 mg/m^3)	2 weeks, 6 hour/day 5 day/week	Cytochrome P-450	content increased only at 750 ppm[f]	Elovaara, 1982
						NADPH-cytochrome c-reductase	activity increased at 400, 750 ppm[f]	
						UDP-glucuronosyl transferase	activity increased all doses[f]	
						Ethoxycoumarin o-deethylase	activity increased all doses[f]	
						Ethoxyresorufin o-deethylase	activity increased all doses[f]	

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[a] All effects significantly ($p<0.05$) different from controls unless otherwise noted.

[b] Hepatic enzyme levels determined in the serum 24 hours postexposure; at 4 hours postexposure levels showed less change than controls.

[c] Increases significant ($p<0.05$) only at higher two dose levels; all others significantly increased at all three dose levels.

[d] Serum cholinesterase levels determined at 4 hours postexposure; levels were below normal by 24 hours postexposure.

[e] 1-hydroxylation of hexane not increased by m- or p-xylene.

[f] Effects noted after 2 weeks of treatment; similar effects were noted after 1 week of exposure for most enzymes.

NS = Not significant

changes in red blood cell metabolism or hepatic adaptation to p-xylene; and increased 5'-nucleotidase may have been due to hepatobiliary dysfunction. The rationale used for determining these suggested causes for the increased enzyme levels was not discussed by the authors (Patel et al., 1979). In several other inhalation studies (Ungvary et al., 1980; Toftgaard and Nilsen, 1981, 1982; Toftgaard et al., 1981), mixed xylene or specific xylene isomers increased hepatic cytochrome P-450, various hydroxylases and dealkylation enzymes following exposure at a minimal concentration of 600 ppm (4 weeks duration, 6 hours/day, 5 days/week) or minimal duration of 3 days (2000 ppm, 6 hours/day). Effects were comparable with those previously noted following oral administration of xylene isomers. Elovaara (1982) reported that, although cytochrome P-450 and NADPH cytochrome c-reductase levels were not significantly affected, inhalation of 50 ppm m-xylene for 2 weeks (6 hours/day, 5 days/week) was effective in significantly ($p<0.05$) increasing hepatic UDP-glucuronosyl transferase, ethoxycoumarin o-deethylase, and ethoxyresorufin o-deethylase activities. These effects were discussed by Elovaara (1982) with respect to a functional adaptive response to m-xylene exposure, metabolism and elimination, and were not considered adverse.

Recently, Toftgaard et al. (1983) examined the amino acid composition and identity of the cytochrome P-450 isozyme induced by xylene. A comparison was made between the xylene-induced and phenobarbital-induced isozymes. Phenobarbital is a more effective inducer of hepatic cytochrome P-450 but xylene and phenobarbital appear to induce a very similar isozyme. Xylene pretreatment (2000 ppm, 6 hours/day for 3 days) increased hepatic cytochrome P-450 from 0.56-1.03 nmoles/mg microsomal protein, while phenobarbital (0.1%

in drinking water for 5 days or 80 mg/kg intraperitoneally for 3 days) increased levels to 1.7 nmoles/mg microsomal protein. Amino acid compositions of both isozymes are nearly identical and, considering slight contamination, they could be the same enzyme. These isozymes have the same molecular weight (54,000 daltons), electrophoretic mobility, immunological reaction, substrate specificity and apparently identical peptide map. Toftgaard et al. (1983) suggested that this isozyme was the same species induced by xylene and phenobarbital, and that this isozyme appears to predicate the formation of 4,5-benzo-α-pyrene epoxide from benzo-α-pyrene. Epoxide formation is of concern, therefore, especially when exposure to several chemicals might be concomitant.

Synergistic/Antagonistic Effects

The effects of concomitant exposure to xylene with various industrial solvents and ethanol have been studied by a number of researchers. Simultaneous exposure to xylene and ethanol has been studied most extensively because of the high potential for workers to be exposed to xylene and to consume alcoholic beverages.

Technical xylene that consisted of 80% m-xylene and 12% o-xylene (300 ppm, 6 hours/day, 5 days/week) was administered to adult male Wistar rats (n=20) for 2 weeks; half of these were simultaneously given 15% ethanol as drinking fluid (Savolainen et al., 1978). The effects of xylene on hepatic and renal enzyme activities (7-ethoxycoumarin o-deethylase, UDP-glucuronosyltransferase and cytochrome P-450) were notably potentiated (more than additively) by simultaneous exposure to ethanol for 2 weeks. Subsequent studies from the same laboratory (Elovaara et al., 1980) exposed rats on the same dose scheme to technical xylene and ethanol for up to 18 weeks. The increased activity of hepatic o-deethylase, 2,5-diphenyloxazole hydroxylase, and UDP-glucuronyltransferase induced by xylene alone (300 ppm, 6 hours/day, 5 days/week) were additively or slightly less than additively enhanced by simultaneous ethanol ingestion (15-20% drinking fluid). An additive effect on renal ethoxycoumarin deethylase activity was noted after xylene-ethanol treatment. Histologically, the livers of rats treated with xylene or ethanol alone were unremarkable, but xylene-ethanol treatment caused increased lipid vesicles within hepatocytes (fatty metamorphosis), referred to as "more severe liver damage."

Experimental studies on the effects of simultaneous exposure to xylene and ethanol have been conducted in human volunteers. Savolainen and Riihimaki (1981b) exposed 10 male subjects to 145 or 290 ppm m-xylene for 4 hours alone and in combination with ethanol (0.8 g/kg). Ethanol was also administered alone at 0.4 and 0.8 g/kg. Testing was conducted once weekly over a period of 9 weeks. The effects of xylene and ethanol on the human equilibrium system were measured by recording body sway with a transducer platform with eyes open and closed (details of equilibrium test in Laboratory Studies in Human Volunteers, Chapter VI). The ratio of body sway with eyes closed:eyes open was used as a measure of nonvisual balance control. Xylene or alcohol alone had little effect on the body sway ratio; in fact, following exposure to 290 ppm xylene, body sway was reduced. Simultaneous exposure to xylene (290 ppm for 4 hours) and alcohol (0.8 g/kg) significantly increased the ratio of eyes closed:eyes open body sway. This indicated an effect on the central equilibrium control mechanisms while visual orientation was not changed (Savolainen and Riihimaki, 1981a). Seppalainen et al. (1981) used the same exposure protocol with 8 male volunteers to investigate the effects of combined xylene (700 or 1200 mg/m^3 for 4 hours) and ethanol (0.4 or 0.8 g/kg) on visual and somatosensory evoked potentials and electroretinograms. No effects on electroretinograms were demonstrated following any exposure and changes in the somatosensory evoked potentials were minor and not dose-related. Alcohol caused latencies in the visual evoked potentials and simultaneous xylene tended to counteract this effect. Xylene alone at 1200 mg/m^3 and xylene at 700 mg/m^3 with ethanol (0.8 g/kg) caused slight decreases in amplitudes of visual evoked potentials. Alcohol alone did not affect the amplitude of potentials. Xylene at 1200 mg/m^3 and simultaneous ethanol ingestion significantly decreased the amplitudes of visual evoked potentials.

The toxicokinetic interactions of m-xylene and ethanol were examined to elucidate a possible mechanism of the additive or potentiational effect of simultaneous exposure (Riihimaki et al., 1982b). Both compounds seem to be metabolized by hepatic alcohol and aldehyde dehydrogenase. Fourteen volunteers were exposed to xylene at 640 or 1220 mg/m^3, with or without ethanol (0.8 g/kg), or ethanol alone. Groups of 4-5 volunteers were exposed to different combinations of xylene or ethanol once weekly over a 5-week period. Simutaneous exposure to xylene and ethanol caused blood levels of xylene to be increased up to 2-fold compared with that following xylene-only exposures, and methylhippuric acid excretion was delayed for several hours. Blood ethanol levels were not affected by simultaneous xylene exposure but xylene did appear to induce increased acetaldehyde formation from ethanol. Two individuals became nauseated following xylene exposure with ethanol ingestion, while neither developed similar symptoms following exposure to either chemical alone. These authors (Riihimaki et al., 1982a,b) discuss the obvious metabolic inhibition of xylene by ethanol and suggest that microsomal oxidation of xylene is delayed. The effect of xylene on ethanol metabolism may also cause alterations so that acetaldehyde-blood levels are elevated and ethanol intolerance develops (Riihimaki et al., 1982b).

The effect of simultaneous m-xylene (200 ppm) and 1,1,1-trichloroethane (a solvent used for cleaning metals and plastic molds) (400 ppm) exposure for 4 hours on human equilibrium was examined in a group of 9 volunteers (Savolainen et al., 1982b). The ratio of body sway with eyes closed:eyes open was measured as previously noted (Savolainen and Riihimaki, 1981b). In this study (Savolainen et al., 1982b) both xylene and 1,1,1-trichloroethane when administered alone at 200 ppm tended to decrease the ratio of body sway. 1,1,1-Trichloroethane at 400 ppm alone caused an initial decrease followed by a sharp increase in the body sway ratio during the last half of the exposure interval. Following simultaneous exposure, the body sway ratio was increased throughout the exposure period, indicating impaired nonvisual equilibrium control. The blood levels of both solvents were doubled during simultaneous exposure.

In a brief communication, Tatrai et al. (1979) exposed rats to xylene and carbon tetrachloride and conducted histological, histochemical and ultrastructural analyses for hepatic damage. Rats were treated with xylene (route not specified) for 4 days, at which time CCl_4 was given orally (2 mℓ/kg bw). Animals were sacrificed on day 5. Xylene potentiated the hepatotoxic effects of CCl_4. Centrilobular necrosis was submassive in animals exposed to both chemicals, and the area of damage was increased

2-fold compared with animals treated with CCl_4 alone. This potentiation was due to metabolic induction by xylene which increased the amount of active CCl_4 metabolites.

In an English abstract of a Japanese study, Nakajima and Sato (1979) reported that simultaneous exposure to m-xylene and toluene or m-xylene and benzene caused metabolic interaction in rats. Benzene metabolism was inhibited by xylene, while toluene and xylene had similar inhibitory effects. Similarly, Gut (1981) reported in an abstract that xylene (212 mg/kg) inhibited benzene metabolism in rats treated concomitantly with mixed xylene and the second solvent. Elovaara et al. (1982) reported that the hepatic metabolism of ethylbenzene was delayed in rats exposed simultaneously to m-xylene. Ethylbenzene, however, had no apparent inhibitory effect on the hepatic metabolism of m-xylene.

Phenobarbital pretreatment in humans and rats was determined to have little effect on the absorption or excretion of m-xylene or m-methylhippuric acid, respectively, when exposure levels were low (400 mg/m³ for 8 hours) (David et al., 1979). In rats exposed to high levels of m-xylene (2000 and 4000 mg/m³ for 6 hours), however, pretreatment with phenobarbital (50 mg/kg/day orally for 3 days) caused an increase in the amount of m-xylene absorbed and m-methylhippuric acid excreted. David et al. (1979) suggested that at low levels of m-xylene exposure nonpretreated humans or animals could metabolize and excrete m-xylene at a rate equal to or above the absorption rate so that blood levels never reached equilibrium with alveolar concentration. At higher levels, however, it became apparent that hepatic metabolism in control animals was rate limiting, and phenobarbital induction allowed more rapid metabolism and excretion.

Summary

Effects of exposure to xylene have been noted in the CNS, lungs, kidneys and liver of experimental animals and the available human studies. Effects on the CNS are usually the first observable signs of toxicity following acute exposures. CNS effects stem from xylenes lipophilic nature and the high lipid content of myelin and nerve axon membranes. The mechanism of xylene neurotoxicity may be one of physical toxicity (saturation of nerve cells to a nonfunctional level), fluidization and altered physiology of the axon membrane or neurotransmitter inhibition. The exact mechanism has not been elucidated and xylene or an active metabolite may be the toxic agent. The central vestibular system, which controls body balance and equilibrium, has been suggested to be the most sensitive region of the brain to xylene intoxication.

Effects on the lung have been attributed to irritation and disruption of the endothelium and alveolar epithelium. Pulmonary metabolism is not as complete as that in the liver and tolualdehyde may be a cytotoxic intermediate in the lung tissue.

Toxic effects in the kidney appear to be attributed to the disruption and altered permeability of the epithelial cells in the glomerulus or renal tubules or both. Possibly because of fluidization of the cell membrane or disruption of the cells that are due to irritation, the permeability of the glomerulus or tubular epithelium increases so that components of the blood (albumin, cells) appear at higher levels in the urine.

Effects of xylene on the liver have been described as being due primarily to functional adaptation to xenobiotic metabolism. Ultrastructural studies (Bowers et al., 1982; Tatrai et al., 1981) have reported proliferation of the smooth endoplasmic reticulum and hepatocyte changes consistent with microsomal enzyme induction and increased detoxication capacity. A number of studies have reported xylene as an inducer of hepatic microsomal enzymes, but the available studies do not report cellular damage that is due to metabolites. Detoxication of xylene to methylhippuric acid or other nontoxic metabolites appears to be fairly complete (Smith et al., 1982).

The effects of simultaneous exposure to xylene and several industrial solvents and ethanol have been studied. Ethanol appears to potentiate the enzyme induction potential and effect on body balance control caused by xylene alone, and may cause increased hepatic damage (Elovaara et al., 1980; Savolainen and Riihimaki, 1981a). Xylene also seems to lower ethanol tolerance (Riihimaki et al., 1982a). Combined exposure to xylene and 1,1,1-trichloroethane caused a sharp increase in body sway, while separately each compound tended to decrease body sway at the exposure levels tested (Savolainen et al., 1982b). Xylene potentiated carbon tetrachloride hepatotoxicity through enzyme induction, which increased the production of the toxic carbon tetrachloride metabolite (Tatrai et al., 1979). Toluene administered concomitantly with xylene delayed xylene metabolism and excretion as methylhippuric acid, but the overall output of this urinary metabolite was not changed (Ogata and Fujii, 1979). Xylene, however, appears to equally inhibit toluene metabolism. Benzene metabolism was apparently inhibited by simultaneously administered m-xylene (Nakajima and Sato, 1979). Ethylbenzene metabolism was delayed in rats when exposure was also to m-xylene (both are components of mixed xylene) (Elovaara et al., 1982).

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the noncarcinogenic and carcinogenic effects. In the quantification of noncarcinogenic effects, an ADI is calculated. The AADI and HA values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{\text{(NOAEL or LOAEL in mg/kg/day) (body weight in kg)}}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking water volume in } \ell\text{/day}} = \text{mg/}\ell$$

where:

NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
Body weight = 70 kg for adult or 10 kg for child
Drinking water volume = 2 ℓ/day for adults or 1 ℓ/day for children
Uncertainty/Safety Factor = 10, 100 or 1000

Utilizing these equations, the following drinking water concentrations are developed for noncarcinogenic effects:

1. A 1-day HA for 10 kg child
2. A 1-day HA for 70 kg adult
3. A 10-day HA for 10 kg child
4. A 10-day HA for 70 kg adult
5. A lifetime AADI for a 70 kg adult

The distinctions made between the HA calculations (items 1-4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1-2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon noncarcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivities to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS, 1977, 1980) are typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.
- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.
- An uncertainty factor of 1000 is used when limited or incomplete acute or chronic toxicity data are available or when only the acute or chronic toxicity data identify a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines (NRC, 1977) for selection of an uncertainty factor, but must be considered, include the following: 1) the quality of the toxicology data, 2) the significance of the adverse effect, and 3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen, mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical by drinking water. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for noncontinuous animal feeding, nonlifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 ℓ of water per day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as 1) incomplete knowledge concerning the health effects of contaminants in drinking water, 2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results, and 3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Existing Guidelines and Standards

NAS (1980) reported a SNARL (suggested no adverse response level) for xylenes in drinking water. The NAS-SNARL value was 21 mg/ℓ xylene for a 1-day exposure and 11.2 mg/ℓ for a 7-day exposure (for a 70 kg adult).

EPA-ODW (1981) also provided HA for a 1-day, 10-day and longer exposure of xylene in drinking water. These HAs for a 10 kg child were 12.0 mg/ℓ, 1.2 mg/ℓ and 0.62 mg/ℓ of xylene for 1-day, 10-day and longer-term exposure, respectively.

The American Conference of Governmental Industrial Hygienists (ACGIH, 1982) adapted a TLV for xylenes in workplace air of 435 mg/m³ as a TWA for an 8-hour workday.

Noncarcinogenic Effects

Based on the median lethal dose levels of xylene administered orally or by inhalation (see Table V-1), xylene has a relatively low toxicity to animals and has even been defined as "nontoxic" or "relatively harmless" (Hine and Zuidema, 1970). Oral LD_{50} values for o-, m- and p-xylene were 3.6, 5.1 and 4.1 g/kg, implicating o-xylene as the most toxic isomer (Ungvary et al., 1979). Bonnet et al. (1979) suggested that p-xylene was the most toxic isomer when administered by inhalation. LC_{50} values for 6 hours of exposure were 3907, 4595 and 5267 ppm, respectively, for p-, o- and m-xylene.

In a study of the toxic effects of o-, m- and p-xylene following oral administration, Ungvary et al. (1981b) reported that female rats (CFY strain) were more sensitive than males. Doses of 2.2 g/kg/day for 4 days caused ~20% mortality among females after exposure to any isomer, while no males died. Toxic effects among survivors included decreased body weight gain, spleen and thymus weights and increased liver weights with centrilobular hepatocyte hypertrophy. Pyykko (1980) exposed male rats to 1.1 g/kg/day of all three isomers for 3 days. Toxic effects at this dose level were less extensive and included significant weight loss ($p<0.05$) and increased liver weights (o- and m-xylene treatment, only). In rabbits, a single dose of 1.3 g/animal of any xylene isomer caused no grossly apparent toxic effects. At 1.75 g/animal, m- and p-xylene were also well tolerated, but o-xylene caused high levels of urinary "reducing material" (Bray et al., 1949).

In a recent teratogenicity and embryotoxicity study (Marks et al., 1982), mixed xylene was administered by gavage in cottonseed oil to pregnant CD-1 mice on days 6 through 15 of gestation (see Teratogenicity Section in Chapter V). Administered doses ranged from 0.52-4.13 g/kg/day. Maternal mortality was reported at doses >2.58 g/kg/day. Cleft palates, decreased fetal weights and maternal hepatotoxicity were evident at $\geq$2.06 g/kg/day. No toxic effects on the dams or fetuses were demonstrated at below 1.03 g/kg/day following 9 days of treatment.

Inhalatory exposure to mixed xylene at 2500 mg/m^3 (575 ppm) for 4 hours caused no distress or signs of gross toxicity among rats (Carpenter et al., 1975). Internal or histological examinations of these animals were apparently not conducted. Batchelor (1927) reported that a level of 620 ppm was tolerated with slight, if any, toxicity in rats exposed for 18-20 hours/ day for up to 7 days. Gross and pathological examinations revealed no symptoms of toxicity and body weight gain was 3% greater than contols. At a level of 980 ppm for a similar duration, effects on the nervous system (instability, incoordination, muscular twitching) and kidneys (acute congestion and cloudy swelling) were reported.

In a study testing the embryotoxic and maternotoxic effects of the xylene isomers, Ungvary et al. (1980b) reported adverse effects at levels as low as 1500 mg/m^3 (350 ppm). Pregnant CFY rats were exposed at 150, 1500 or 3000 mg/m^3 for 24 hours/day on days 7-14 of gestation. Relative liver weight was increased in dams exposed to all levels of o-xylene. o-Xylene at 1500 and 3000 mg/m^3 caused significantly decreased ($p<0.001$) fetal weights. The m- and p-isomers of xylene caused maternal (decreased weight gain, reduced number of implantations/dam and mortality) and embryotoxic effects (reduced weight, resorption and skeletal retardation) only after exposure at 3000 mg/m³ (700 ppm) (Ungvary et al., 1980). In a subsequent study, Ungvary et al. (1981b) reported decreased fetal weights in rats treated continuously at 3000 mg/m³ for 48 hours. These effects may have been due to decreased peripheral estradiol and progesterone levels, which led to retarded fetal development. Delayed skeletal development was suggested in fetuses of dams exposed to mixed xylene at 1000 mg/m³ on days 9-14 of gestation (24 hours/day) (Hudak and Ungvary, 1978).

In a 2-week study, brain glutathione levels were decreased and NADPH-diaphorase activity was increased in rats treated with m-xylene at 212 mg/m³ (50 ppm, 6 hours/day, 5 days/week) (Savolainen and Pfaffli, 1980). At higher levels (1710 and 3220 mg/m³) m-xylene additionally decreased brain superoxide dismutase levels and increased azoreductase activity.

In humans, subjective complaints primarily of mucous membrane irritation were reported in males and females exposed to p-xylene at 100 ppm and 150 ppm for 7.5 hours/day on 5 consecutive days (Hake et al., 1981). No observable toxic effects were reported for neurological function (equilibrium, EEG) cardiopulmonary function or cognitive testing performance. These authors concluded that 100 ppm (7.5 hours/day for 5 days) was near a NOEL for mucous membrane irritation in sedentary males exposed by inhalation. Carpenter et al. (1975) also reported no significant effects in subjects exposed to 460 mg mixed xylene/m³ (110 ppm) for 15 minutes. Mixed xylene at 1000 mg/m³ (460 ppm) and 2000 mg/m³ (690 ppm), however, caused eye irritation and dizziness in 1/7 subjects and 4/6 subjects, respectively.

CNS function, measured by various cognitive and performance tests, was affected only after exposure to 1300 mg/m³ mixed xylene (300 ppm) in conjunction with excercise (Gamberale et al., 1978). Savolainen et al. (1980a) reported that nonvisual control of body balance was impaired by m-xylene at 90 ppm (6 hours/day) on the first day of exposure, but tolerance to the xylene developed. Choice-selection-reaction time and flicker-fusion threshold frequency were impaired in subjects exposed at 280 ppm m-xylene for 4 hours, but no effect on these parameters was evident following exposure at 160 ppm (Savolainen, 1980; Savolainen et al., 1980b).

The available reports of cases of acute xylene poisoning are of little value for quantification of xylene toxicity, since exposure levels are not available and effects are often severe.

A number of inhalation studies of 30-90 days duration have been conducted on rats, guinea pigs, beagle dogs and squirrel monkeys (see Table V-2). No oral studies of this duration were available. Jenkins et al. (1970) exposed Sprague-Dawley rats, Princeton guinea pigs, beagle dogs and squirrel monkeys to o-xylene at 337 mg/m³ (78 ppm) continuously for 90 days or at 3358 mg/m³ (772 ppm) for 8 hours/day, 5 days/week for 6 weeks. Three of 12 rats exposed at 3358 mg o-xylene/kg died during the exposure. No effects on body weight gain or hematology were reported for any species exposed on either dosing schedule. Carpenter et al. (1975) exposed rats and beagle dogs to mixed xylene at 770, 2000 and 3500 mg/m³ (180, 460 and 810 ppm) for 6 hours/day, 5 days/week for 13 weeks duration. No effects on body weight gain, hematology, blood chemistry, kidney or liver weights or tissue histology were reported for either species.

In an early inhalation study (Smyth and Smyth, 1928) guinea pigs exposed at 300 ppm (1300 mg/m^3) m-xylene for 4 hours/day, 6 days/week for 11 weeks developed slight degeneration of the liver and pulmonary inflammation. In repeating the study, exposed guinea pigs (same concentration and dosing schedule, but for 10 weeks duration) developed moderate renal tubular degeneration and pulmonary inflammation.

Decreased body weight, hepatomegaly and ultrastructural hepatocyte changes were reported in rats exposed by inhalation at 3500 ppm (8 hours/day) for 6 weeks (Tatrai and Ungvary, 1980). No mortality was reported.

Only one study >90 days duration in which xylene was administered orally was available for review. Male Long-Evans rats were fed a diet containing 200 mg o-xylene/kg food for up to 6 months, and their livers were examined grossly and ultrastructurally for toxic effects (Bowers et al., 1982). Livers of all exposed animals appeared normal after gross inspection. Ultrastructurally, hepatocytes of rats treated with o-xylene or other methylated benzenes developed two types of vesicles not seen in control rats. One vesicle type appeared to originate from the smooth endoplasmic reticulum, while the second was associated with the plasmalemma. Both vesicle types could be correlated to increased hepatic metabolism of xylene or the administered methylbenzene. Both vesicle types appeared within 1 month of treatment but did not increase during longer exposure periods (Bowers et al., 1982).

Toxic effects have been reported in male CFY rats following inhalation exposure to o-xylene at 4750 mg/m³ for 8 hours/day, 7 days/week for 1 year (Tatrai et al., 1981). Activity of several hepatic enzymes (cytochrome P-450, cytochrome b_5, NADPH-cytochrome c reductase, aniline hydroxylase, aminopyrin-N-demethylase) was increased in exposed rats. Body weight gain was decreased despite increased intake of water and food. Hepatomegaly was reported. Hepatic tissue appeared normal upon histological examination, but ultrastructurally hepatocytes had proliferation of smooth endoplasmic reticulum, glycogen depletion, mitochondrial changes and increased numbers of autophagic bodies and peroxisomes. These effects were also regarded as evidence of functional adaptation for xylene metabolism.

In humans, epidemiological reports suggest renal dysfunction as a possible effect of chronic occupational exposure to xylene and other solvents. Askergren and coworkers (Askergren et al. 1981a,b; Askergren, 1981, 1982) examined a group of 40 paint industry workers exposed to xylene and toluene. Exposure levels were generally below Swedish standards (350 mg/m³ for xylene; 300 mg for toluene), but occasionally exceeded these limits by 2-3 times. Urinalyses were used to reveal an increased number of exposed subjects who excreted high levels of albumin and blood cells. These symptoms were diagnosed as indicative of increased glomerular permeability and possible basement membrane damage. In another study of painters (n=118) exposed to toluene, xylene and other benzene homologues (exposure levels not reported), Franchini et al. (1983) reported that urinary albumin levels were not elevated but β-glucuronidase levels were significantly increased compared with controls. Effects suggested renal tubular damage but were regarded as minimal.

A group of 102 car painters exposed to several paint solvents (toluene 30.6 ppm, xylene 5.8 ppm, butyl acetate 6.8 ppm) were studied to determine the effects of the solvents on serum levels of hepatic enzymes, neurophysiological functions and ophthalmological parameters (Kurppa and Husman, 1982). A group of 102 age-matched locomotive engineers and assistants were used for comparison. Lens opacities were identified in 48/92 painters having ophthalmological examinations. Twelve of 59 painters examined for nerve function had abnormally slow motor and sensory conduction velocities and long neuromuscular latencies. Levels of serum enzymes were comparable between the painters and the referral group. It is difficult to attribute any of these effects to xylene, since exposure was to many solvents and xylene was not the major component.

Quantification of Noncarcinogenic Effects

The toxic potencies of the three xylene isomers are somewhat different, as evident from LD_{50} levels (see Table V-1). The lack of appropriate data for each isomer does not allow separate quantification; therefore, HA levels reported here for drinking water will be presented for xylene in general. The HA levels may be based on toxicity data of one specific isomer, but applied uncertainty factors allow the HAs to be used for all isomers, mixed or technical xylene.

1-Day Health Advisory. In the absence of experimental data utilizing the oral route of administration of xylenes, the inhalation data may be considered in the derivation of a 1-day HA for xylenes. Gamberale et al. (1978) performed two sets of experiments utilizing human volunteers. In the first set, 15 male subjects inhaled 435 and 1300 mg/m^3 xylenes at rest.

No adverse health effects were observed in the first set of the experiments. Xylenes uptake after 435 and 1300 mg/m^3 exposure was estimated to be 180 and 540 mg. In the second set, 8 of these 15 subjects were exposed to 1300 mg/m^3 xylene in inspired air; the exposure period started with 30 minutes of work on a bicycle ergometer and continued during behavior tests. Each exposure period lasted 70 minutes. The volunteers were subjected to tests of numerical ability, reaction time, short-term memory and critical flicker fusion. The authors concluded that there was evidence of reduction in the performance on 3 of the 4 tests in the second set of experiments. In this set of experiments the average uptake of xylenes was estimated as 1200 mg. An inhalation concentration of 1300 mg/m^3 xylene (Gamberale et al., 1978) may be used as a NOAEL in humans for the determination of a 1-day HA for drinking water. The 1-day HA may be derived as follows:

For 70 kg adult:

$$\text{1-day HA} = \frac{(1300\ \text{mg/m}^3)\ (1\ \text{m}^3\text{/hour})\ (1\ \text{hour/day})\ (0.64)}{(10)\ (2\ \ell\text{/day})\ (1.0)} \sim 42\ \text{mg}/\ell$$

For 10 kg child:

$$\text{1-day HA} = \frac{(1300\ \text{mg/m}^3)\ (1\ \text{m}^3\text{/hour})\ (1\ \text{hour/day})\ (0.64)\times(10\ \text{kg})}{(70\ \text{kg})\ (10)\ (1\ \ell\text{/day})\ (1.0)} \sim 12\ \text{mg}/\ell$$

Where: 1300 mg/m^3 = no-observed-adverse-effect level
1 m^3/hour = assumed amount of air inhaled per hour
1 hour/day = approximate duration of exposure
0.64 = absorption factor for inhalation exposure
70 kg = average body weight of an adult
10 kg = assumed body weight of a child
10 = uncertainty factor for intraspecies extrapolation
2 ℓ/day = assumed consumption of water by an adult per day
1 ℓ/day = assumed consumption of water by a child per day
1.0 = absorption factor for oral exposure

In comparison, the oral administration studies in rats (Pyykko, 1980) and mice (Marks et al., 1982) show minor effects at doses near 1.0 g/kg/day. Pyykko (1980) reported slightly increased liver weights in rats treated by gavage at 1.1 g/kg/day for 3 days. Marks et al. (1982) reported no toxic effects in pregnant mice treated at 1.03 g mixed xylene/kg/day on days 6-15 of gestation, but indicated a trend toward increased fetal resorptions following treatment at ≥2.06 g/kg/day. These data suggest that 1.0 g/kg/day by oral ingestion is near a NOAEL. Using an uncertainty factor of 100 for conversion from an animal NOAEL to the most sensitive humans, the 1-day HA for adults would be 350 mg/ℓ [i.e., (1000 mg/kg bw/day x 70 kg) ÷ (100 x 2 ℓ/day)].

This value is notably higher than the value of 42 mg/ℓ derived previously. The difference may be due partially to humans being a more sensitive species than laboratory animals, but is also probably due to the difference in the route of administration and the endpoint of observed effects. It is obvious that the human subjects identified effects not measurable in the oral administration animal studies. The 1-day HA values of 42 and 12 mg/ℓ for an adult and child, respectively, based on inhalation data represent conservative levels for drinking water. However, it should be noted that an estimated concentration for detection by taste and odor in surface waters is 0.3-1.0 mg/ℓ (Middleton et al., 1958).

10-Day Health Advisory. The study by Carpenter et al. (1975) appears to be the most appropriate basis for a 10-day HA. Groups of male rats (n=25/dose level) were exposed by inhalation to mixed xylene at 770, 2000 or 3500 mg/m^3 for 6 hours/day on 5 days/week for up to 13 weeks (65 exposures). Interim sacrifices were conducted after 3 and 7 weeks of exposure.

Examinations for toxic effects were quite extensive and included gross changes (body weight, appearance), hematological and blood chemistry (hematocrit, erythrocyte, reticulocyte, differential, leukocyte counts and serum alkaline phosphatase, SGPT, SGOT and BUN) and tissue histology (lung, liver, kidney, trachea, adrenal, brain, pituitary, thyroid, parathyroid, heart, spleen, stomach, duodenum, pancreas, small intestine, colon, skeletal muscle, sciatic nerve and bone marrow). Eosinophil and monocyte counts appeared to be increased after 3 weeks of exposure at 3500 mg/m³; this trend was not seen at 7 and 13 weeks. No significant effects on blood chemistry or tissue histology were reported as a result of xylene exposure. One rat treated at 3500 mg/m³ for 7 weeks showed slight renal tubular regeneration, and at 13 weeks, the response was noted in rats in a nondose-related manner. The Harlan-Wistar strain of rats was felt to inherently show tubular regeneration; this response was not considered dose-related (Carpenter et al., 1975). Based on the minor effects noted following inhalation exposure at 3500 mg/m³ mixed xylene (6 hours/day, 5 days/week), a level of 2000 mg/m³ will be regarded as the NOAEL. A 10-day HA may be derived as follows:

For 70 kg adult:

$$\text{10-day HA} = \frac{(2000\ \text{mg/m}^3)\ (6\ \text{hours/day})\ (1\ \text{m}^3/\text{hour})\ (5/7)\ (0.64)}{(100)\ (2\ \ell/\text{day})\ (1.0)} \sim 27\ \text{mg}/\ell$$

For 10 kg child:

$$\text{10-day HA} = \frac{(2000\ \text{mg/m}^3)\ (6\ \text{hours/day})\ (1\ \text{m}^3/\text{hour})\ (5/7)\ (0.64)\ (10\ \text{kg})}{(100)\ (70\ \text{kg})\ (1\ \ell/\text{day})\ (1.0)}$$

$$= 7.8\ \text{mg}/\ell$$

where: 2000 mg/m^3 = no-observed-effect level
6 hours = number of hours exposed in a day
1 m^3/hour = assumed amount of air inhaled per hour
5/7 = fraction converting from 5- to 7-day exposure
0.64 = absorption factor for inhalation exposure
10 kg = assumed body weight of a child
100 = uncertainty factor for both intra- and interspecies extrapolation
70 kg = assumed body weight of an adult
1 ℓ/day = assumed consumption of water by a child per day
2 ℓ/day = assumed consumption of water by an adult
1.0 = absorption factor for oral exposure

It should be noted that these HA levels also exceed the taste and odor level of 0.3-1.0 mg/ℓ.

Lifetime AADI. An ingestion study by Bowers et al. (1982) in aged rats or one of several inhalation studies in various animal species could be used as a basis for the lifetime AADI.

The study by Bowers et al. (1982) was designed primarily to investigate the first hepatocyte changes following long-term exposure to low levels of o-xylene or other methylbenzenes. o-Xylene was administered to aged male rats, weighing 800-900 g, in the diet at 200 mg/kg food for up to 6 months prior to electron microscopic examination of their livers. Ultrastructurally, hepatocytes of rats ingesting the test compound in the diet (200 mg/kg diet) developed two types of vesicles not present in control animals within 1 month of treatment. Both vesicles, one associated with the smooth endoplasmic reticulum and the other associated with the plasmalema, could be interpreted as structural evidence of enzyme induction and increased hepatic metabolism of xylene. However, histological examination of tissues other than liver was not performed by electron microscopy. The appearance of these two vesicle types in hepatocytes was not stated specifically for o-xylene, but was reported for all methylbenzenes tested in general.

In an inhalation study by Jenkins et al. (1970), o-xylene was administered by inhalation to rats, guinea pigs, monkeys and dogs for 30 repeated exposures, 8 hours/day, 5 days/week or 90 days continuous exposure. The dosage for 30 repeated exposures and 90 days continuously were 3358 and 337 mg/m^3, respectively. During the 3358 mg/m^3 o-xylene repeated study, two rats died on the third exposure day and another rat and one monkey died on day 7; one of the dogs exhibited tremors of varying severity throughout. Besides the above mentioned observations, no significant effects were observed with respect to body weight, hematology and histopathological examination at either dose. Another inhalation study by Carpenter et al. (1975) could be used as the basis for the AADI. However, this study, when compared with Jenkins et al. (1970) did not test as many species nor expose the animal continuously.

The evaluations of the above studies, Bowers et al. (1982) and Jenkins et al. (1970), suggest that even though the study by Bowers et al. (1982) was conducted in rats by the ingestion route of exposure, certain major weaknesses of this study rule out its consideration in the development of lifetime AADI. These weaknesses are: 1) the use of aged animals weighing 800-900 g in the experiment; 2) stability of o-xylene was not monitored (i.e., any loss due to evaporation not mentioned); 3) single exposure level; 4) absence of histological examination of tissues other than liver of animals on test diet; and 5) ultrastructural changes in the hepatocytes of rats ingesting o-xylene was not stated specifically for o-xylene.

Therefore, the inhalation study by Jenkins et al. (1970) is chosen as the basis for the lifetime AADI. In this study, as stated earlier, an inhalation dose of 337 mg/m³ continuously for 90 days in rats, guinea pigs, monkeys and dogs did not produce any statistically significant effects with respect to body weight, hematology and histopathological examination of treated animals when compared with control animals. using 337 mg/m³ as a NOEL, the ADI for a 70 kg adult is calculated as follows:

$$\text{ADI} = \frac{337\ \text{mg/m}^3 \times 20\ \text{m}^3\text{/day} \times 0.64}{1000} = 4.31\ \text{mg/day}$$

where: 337 mg/m³ = no-observed-effect level

20 m³ = assumed volume of inspired air per day by an adult human

0.64 = absorption factor

1000 = uncertainty factor to account for both intra- and interspecies variability to the toxicity of a chemical and an additional 10-fold for a study of acceptable quality but significantly less than lifetime in duration.

Using 4.3 mg/day as the ADI and assuming the 70 kg adult consumes 2 ℓ of drinking water per day, the AADI is calculated as follows:

$$\text{AADI} = \frac{4.3\ \text{mg/day}}{2\ \ell\text{/day}} -2.2\ \text{mg}/\ell$$

It should be noted that an estimated concentration for detection by taste and odor in surface water was 0.3-1.0 mg/ℓ (Middleton et al., 1958).

A summary of the data used to calculate HAs and AADI in the Noncarcinogenic Section is provided in Table VIII-1.

TABLE VIII-1

Summary of Data Used to Derive HA and AADI Values

Criteria	Species	Exposure Level	Duration	Daily Dose	Effect	Value of HA or AADI		Reference
						Adult	Child	
1-day HA	human	1300 mg/m^3	1 hour	11.88 mg/kg bw/day	NOAEL	42 mg/ℓ	12 mg/ℓ	Gamberale et al., 1978
10-day HA	rat	2000 mg/m^3, 6 hours/day, 5 days/week	13 weeks	78.37 mg/kg bw/day	NOEL	27 mg/ℓ	7.8 mg/ℓ	Carpenter et al., 1975
AADI	rat, dog, guinea pig and monkey	337 mg/m^3 continuously	90 days	61.62 mg/kg bw/day	NOEL	2.2 mg/ℓ	NA	Jenkins et al., 1970

NA = Not applicable

Carcinogenic Effects

The available data on the carcinogenic potential of xylene is limited to the study by Berenblum (1941) in which undiluted xylene was tested as a skin carcinogen. The dose of xylene applied to the skin of 40 white mice was not reported, but four mice died during the treatment period. Xylene was applied to the shaved skin between the shoulder blades once weekly for 25 weeks. Only one animal developed a tumor, which may have been a papilloma, so xylene was not considered to be a skin tumorigen.

A long-term carcinogenicity bioassay on xylene is presently being conducted by the NTP (1983). Mice and rats were treated by group, but data from the study are not yet available. Histopathological examination of animals, compilation of data and a final report are yet to be completed. The carcinogenic potential of xylene will be better assessed when this report is available for review.

Quantification of Carcinogenic Effects

The very limited data suggest that xylene is not a skin tumorigen. Because of the lack of additional data on the carcinogenicity of xylene, an assessment on this basis is not possible.

Special Considerations

Synergistic Effects. Studies on the toxic effects of concomitant exposure to xylene and other industrial solvents and ethanol have revealed potentiating interactions in some cases. Because of the high number of workers exposed to xylene who may consume alcoholic beverages, the interactions between xylene and ethanol after simultaneous exposure have been studied most extensively. In rats, the enzyme induction of hepatic and renal enzymes by xylene was potentiated by ethanol ingestion (Savolainen et al., 1978). Histologically, simultaneous xylene-ethanol treatment caused lipid vesicles within hepatocytes, while either chemical alone caused no visible hepatic changes (Elovaara et al., 1980). The effects of xylene and ethanol on nonvisual body balance control in humans were increased following exposure to both chemicals (Savolainen and Riihimaki, 1981a). The interaction between xylene and ethanol stems from their similar metabolic pathways. Both chemicals require hepatic alcohol and aldehyde dehydrogenase for detoxication and ethanol appears to be preferred (Riihimaki et al., 1982a). Ethanol ingestion simultaneous with xylene inhalation caused increased blood-xylene levels and delayed methylhippuric acid excretion, but blood ethanol levels were not elevated (see Synergistic/Antagonistic Effects Section in Chapter VII). Two of 14 volunteers became nauseated after simultaneous exposure, while neither had any toxic symptoms after single exposures to xylene or ethanol at the same doses.

Exposure to m-xylene and 1,1,1-trichloroethane simultaneously in humans caused decreased nonvisual body balance control (Savolainen et al., 1982b). These two compounds appear to interact more equally, since blood levels of both compounds doubled during concomitant exposure.

Xylene has potentiated the hepatotoxic effects of CCl_4 in rats (Tatrai et al., 1979). Centrilobular necrosis was more severe, and the area of damage was doubled in rats treated with both xylene and CCl_4 compared with those ingesting CCl_4 alone. Metabolic induction of the enzymes that produce the active CCl_4 metabolites by xylene was suggested as the apparent cause for the synergistic effect.

The metabolic interactions between m-xylene and toluene appear to be equal. Ogata and Fujii (1979), Gut (1981) and Nakajima and Sato (1979) reported that, following simultaneous exposure in rats, metabolism and excretion of urinary metabolites of both toluene and xylene were delayed but overall metabolite profiles were not altered. Benzene metabolism to phenol, however, was inhibited by simultaneous xylene treatment in rats (Nakajima and Sato, 1979; Gut, 1981). Ethylbenzene metabolism was delayed by simultaneous m-xylene exposure, but m-xylene metabolism did not appear affected by ethylbenzene in rats (Elovaara et al., 1982). Phenobarbital caused increased absorption of m-xylene and excretion of m-methylhippuric acid in rats exposed at high levels (2000 and 4000 mg/m³ for 8 hours) (David et al., 1979). At low levels of xylene (400 mg/m³) enzyme induction by phenobarbital had no visible effect because hepatic-metabolism was not saturated; therefore, absorption and excretion proceeded at a maximal rate.

High Risk Subpopulations. The number of workers exposed to xylene either in its manufacture or distillation, during its gasoline blending or during its use as a solvent is great. Based on human case reports and epidemiological studies, groups of interest have been laboratory workers, especially histology technicians, and painters or paint industry workers. These workers would be exposed occupationally and environmentally to xylene, and would be at a higher risk of xylene toxicity.

Based on the synergistic interactions between xylene and other chemicals (see Synergistic Effects Section), humans exposed to xylene and ethanol, 1,1,1-trichloroethane, toluene, benzene and ethylbenzene may be at increased risk of xylene toxicity. Simultaneous exposure to CCl_4 and xylene may cause increased risk of hepatic damage by CCl_4.

As suggested by Holmberg and Nurminen (1980), pregnant women and fetuses are considered to be more susceptible to toxic compounds. Based on studies in animals (Marks et al., 1982; Hudak and Ungvary, 1978; Ungvary et al., 1980), xylene exposure during gestation appears to cause maternal and fetal toxicity at relatively low levels.

In fact, the lowest NOAEL for acutely exposed animals was in pregnant mice and fetuses (Marks et al., 1982). Toxic effects included decreased fetal weights, increased resorptions, increased cleft palates, delayed skeletal development and increased maternal liver weight. Ungvary et al. (1981b) suggest that delayed fetal development may be caused by decreased levels of progesterone and estradiol. Titers of these hormones are apparently lowered due to xylene's inductive effect on metabolism, which causes increased hormone catabolism.

Summary

A review of the pertinent noncarcinogenic effects in animals and humans has been presented with emphasis on oral administration and inhalation studies. Xylene has a relatively low toxicity following acute oral or inhalation exposure. An oral dose of near 1.0 g/kg/day for acute (1-14 days) exposures was regarded as a NOAEL in animals (Marks et al., 1982; Pyykko, 1980). In humans, inhalation exposures near 100 ppm (434 mg/m^3) for 7.5 hours were considered to be a NOAEL (Hake et al., 1981). This level was based on mucous membrane irritation and should be quite conservative for preventing adverse toxic affects. The work of Gamberale et al. (1978) suggests that inhalation NOAELs may be higher. One-day HA levels were determined for an adult and child for several of these NOAELs and a comparison made between them. The recommended 1-day HAs were those derived from the NOAEL of 1300 mg/m³ for humans. The respective 1-day HA values for an adult and a child were 42 and 12 mg/ℓ (see Table VIII-1).

Studies of 30-90 days duration were used for the derivation of the 10-day HA. Studies by Carpenter et al. (1975) and Jenkins et al. (1970) exposed several species of laboratory animals (rats, guinea pigs, dogs and monkeys) to xylene by inhalation for 13 or 6 weeks, respectively. No oral administration studies of this duration were available. No-effect levels of xylene in rats were similar in the two studies, but the absorbed daily dose determined from data reported by Carpenter et al. (1975) was slightly more conservative. Rats exposed to mixed xylene at 2000 mg/m³ for 6 hours/day, 5 days/week developed no observable toxic effects even though gross, hematological and histological examinations were performed (Carpenter et al., 1975). Using this as a NOEL, an absorbed daily dose for humans would be 78.37 mg/kg bw/day, assuming 64% absorption. The 10-day HAs for an adult and child were 27 and 7.8 mg/ℓ, respectively (see Table VIII-1).

Chronic exposure data in humans were not sufficient for quantitative risk estimation. However, the study by Jenkins et al. (1970), even though the route of exposure was inhalation in animals, was regarded as appropriate in derivation of the lifetime AADI. In this study, rats, guinea pigs, monkeys and dogs were exposed to o-xylene continuously for 90 days. The NOEL was 337 mg/m³. Bowers et al. reported that ingesting a diet containing 200 mg o-xylene/kg food for 6 months caused ultrastructural hepatocyte changes in aged male rats. These changes were not specifically confined to o-xylene but also to other solvents. In addition, stability of o-xylene in the diet was not stated in terms of its loss due to evaporation. Tatrai et al. (1981) reported decreased body weight, liver enlargement and ultrastructural changes in hepatocytes of rats inhaling o-xylene at 4750 mg/m³ for 8 hours/day, 7 days/week for 1 year. Using 337 mg/m³ as the NOEL (Jenkins et al., 1970), the AADI of 2.2 mg/ℓ was derived.

Available carcinogenicity data regarding xylene is limited to one skin-application study (Berenblum, 1941). This study was negative. Preliminary data or a final report on the carcinogenicity bioassay on xylene, conducted by the NTP (1983), was not available for review at this time. The lack of carcinogenicity data precludes quantification of these effects.

Several industrial chemicals and ethanol have been found to interact with xylene upon simultaneous exposure. Potentiated toxicity stems primarily from the inhibition of xylene, which leads to elevated blood-xylene levels. Xylene can also potentiate to toxic effects of CCl_4 by inducing enzymes, causing an increase in the formation of active metabolites. Because of the metabolic interaction of ethanol and xylene and the prevalence of both compounds, the potentiation of xylene toxicity by ethanol industrially may be most important.

The number of workers exposed to xylene is quite large, and all are at elevated risk of absorbing toxic doses. Workers exposed to xylene and other chemicals suggested of being synergists are at even greater risk of xylene intoxication. In addition to occupationally exposed people, pregnant females and fetuses, another large group, may be at elevated risk because of their increased sensitivity.

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